Department of Forensic Science

CONTROLLED SUBSTANCES PROCEDURES MANUAL

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1 INTRODUCTION

1.1 Introduction

The information in this Procedures Manual was collected from numerous sources and is presented here for easy reference for drug chemists. In all cases, it is acceptable to use the most current edition of listed literature references. This manual presents a basic outline of the types of drugs analyzed, a source book on reagent and standard preparation and a description of the analytical techniques used with a review of instrumentation. This manual is not all-inclusive, and will reference other sources where appropriate. It is always the chemist's responsibility to choose the best analytical scheme for each individual case. It is expected that supervisors will be consulted for extraordinary procedures.

1.2 Examination Documentation

1.2.1 Specific worksheets for use in analytical notes are provided as controlled forms and should be used as designed. Examiners are reminded to take appropriate notes which will allow for another examiner/supervisor to repeat the analysis under conditions as close as possible to the original, evaluate the data, interpret the results and come to the same conclusion.

1.2.2 The General Drug Worksheet is a generic worksheet for controlled substances casework. The comments section should be used for explanations of tests or lists of weights, etc.

1.2.3 The Blank Worksheet is available for cases requiring more notes than will easily fit on the general worksheet. Cases involving tampering, for example, would not be expected to fit on a general form.

1.2.4 The Pharmaceutical Identifiers (PI) Worksheet is designed for tablet and capsule analysis. Tablet/capsule analysis may still be documented on the general worksheet; however, the PI worksheet is useful when cases contain multiple items of tablets and/or capsules.

1.2.5 The UOM Weight Worksheet is designed to record weight calculations relating to the uncertainty of measurement.

1.2.6 Date(s) of examination shall be noted as "Date started" and "Date completed". The completion date reflects the date when all data has been incorporated into a recorded conclusion.

1.3 The Department's laboratory facilities provide sufficient environmental conditions to conduct all tests listed in the Procedures Manual with no further consideration required.

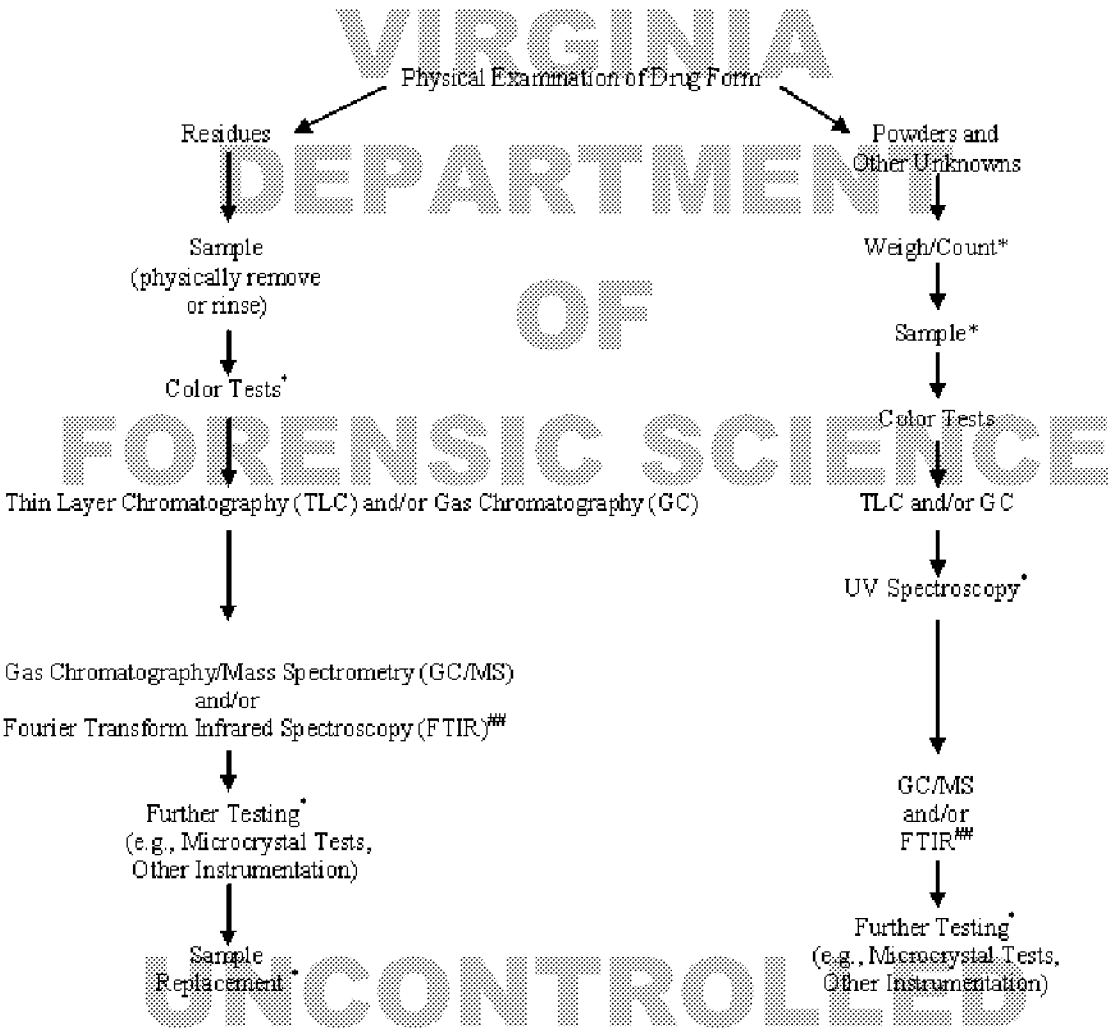
1.4 New procedures must be validated before use. Published procedures must be verified to work in each Regional Laboratory before use. Prior to beginning a validation process, consult the Chemistry Program Manager and the SWGDRUG guidelines for an appropriate validation plan.

2 ANALYTICAL SCHEMES

2.1 Introduction

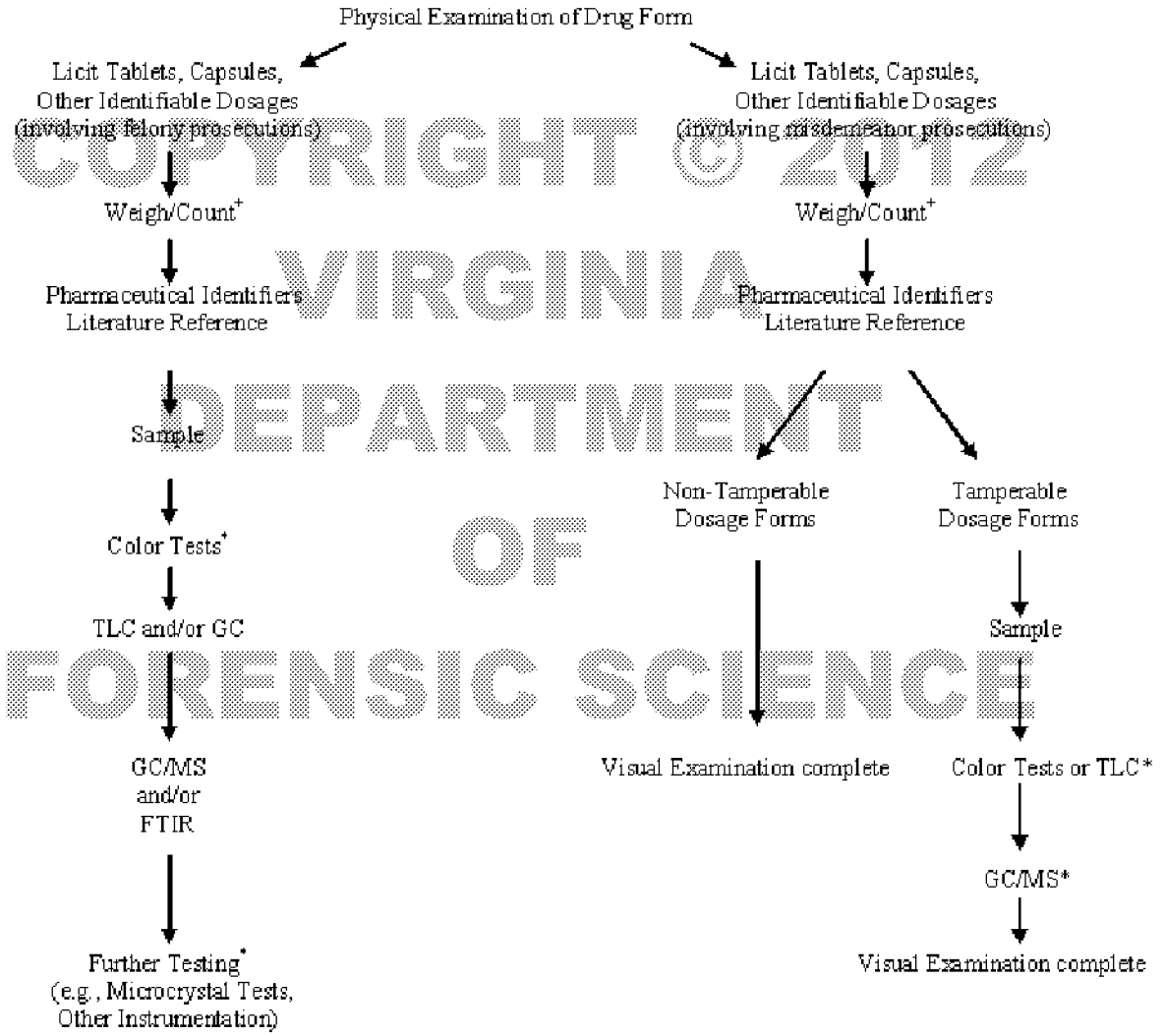
There are three general analytical schemes to be used for controlled substances. At various times, a drug chemist will encounter drug substances for analysis that require specialized analysis. For these cases the flowchart for general unknowns can be followed and any modifications must be approved by the Section Supervisor or Chemistry Program Manager as per ¶ 5.3.10 of the Quality Manual. It should be noted that sample size or other circumstances may require a rearrangement or modification of one or more steps.

2.2 General Unknowns/Powders/Illicit Tablets



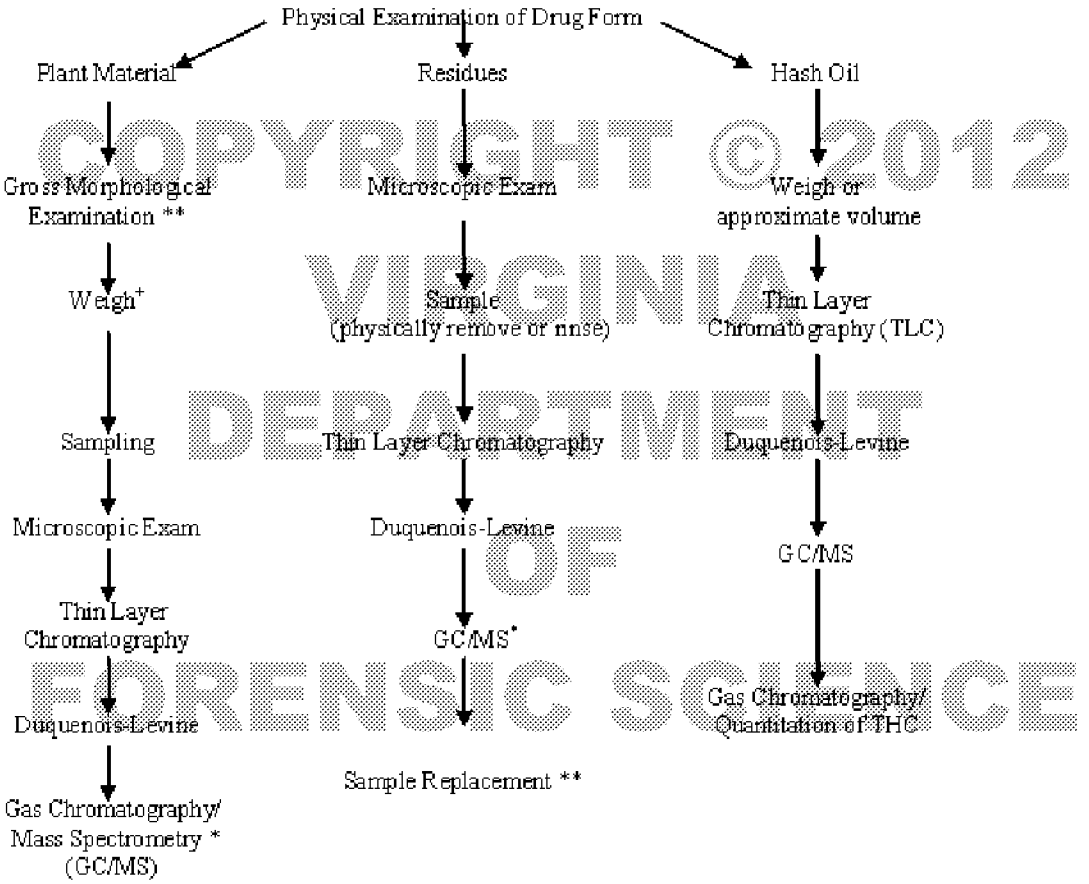
*As appropriate
Or other appropriate definitive structural elucidation method

2.3 Tablets and Capsules



+ As appropriate (dosage forms not generally weighed)
* As Needed

2.4 Marijuana



+ Gross weight suitable if less than ½ ounce with innermost packaging or simple possession

* Required if microscopic characteristics are absent or if another test is inconclusive (see ¶ 6.6.1)

** As needed

2.5 Pharmaceutical Identifiers

2.5.1 Check the *Physician's Desk Reference*, Poison Control, *DEA Logo Index*, *Ident-a-drug*, *Drug ID Bible* or other similar sources for information relating to inscriptions on tablets and capsules, size, color and shape. Two unrelated references are recommended for unfamiliar tablets if no further analysis will be conducted.

2.6 Color Tests

2.6.1 If the size of the sample is sufficient, perform the appropriate color tests required to provide an indication of any compounds present.

2.6.2 Check the available literature (e.g., Clarke) for the interpretation of results of these and/or ask other chemists, as necessary.

2.7 Chromatography

2.7.1 Dissolve the sample directly into a suitable solvent (e.g., methanol). If appropriate, extract the sample from an acidic or basic medium (or both if the contents of the sample are still unknown at this time).

2.7.2 If sample is an unknown, run appropriate screening systems via TLC and/or GC.

2.7.2.1 For TLC, if there are no spots visible under UV light or with visualization sprays, the examiner may halt the analysis and report "No controlled substances found". The examiner may continue with the analytical scheme for any number of reasons to include information provided on the RFLE or other clues from the sample.

2.7.2.2 For GC, if there are no peaks are present, the examiner may halt the analysis and report "No controlled substances found". The examiner may continue with the analytical scheme for any number of reasons to include information provided on the RFLE or other clues from the sample.

2.7.3 If sample identity was indicated previously, choose the appropriate two system TLC and/or two system GC systems, as needed, with a standard. The choice of TLC or GC should be based on which is most appropriate under the circumstances.

2.7.4 In addition to two system TLC, sample retention times obtained from GC/MS systems are often compared to standard retention times.

2.8 Ultraviolet Spectroscopy

2.8.1 An ultraviolet scan can be run on extracted samples or directly on the material itself, if it is pure enough and contains no interfering substances, as a screening test. Suggested solutions are 0.1N HCl, 0.2N H_2SO_4, 0.1N NaOH or Ethanol.

2.8.2 Ultraviolet spectroscopy can be used for quantitative comparisons in cases involving product tampering.

2.9 Infrared Spectroscopy/Mass Spectrometry

2.9.1 If the identity of the sample is still unclear at this point, the IR or GC/MS will provide further information.

2.9.2 The AccuTOF-DART screening method provides accurate mass information, which may assist in identifying components present in the sample. This screening method can be useful at any point in the analytical scheme.

2.9.3 A definitive structural identification technique such as GC/MS or IR is required to be used on all substances where the identities will be reported.

2.10 Further Testing

2.10.1 If the sample is still an unknown or other confirmation is needed, the chemist should use any instrumental techniques available (or combinations thereof) to arrive at a sound analytical conclusion about the identities of sample. This may involve using Department Instrument Support as approved by the Chemistry Program Manager.

2.10.2 Microcrystal tests are used for isomer determination only. They are to be used only in combination with a structural elucidation technique.

2.11 Liquids

2.11.1 Liquid samples may be submitted for analysis as part of a clandestine lab prosecution or for general drug

analysis. These types of samples may require additional steps in the general analytical scheme in ¶ 2.2. Occasionally, it will be necessary to consult with a supervisor, the investigating officer, or both to determine the best analytical course.

2.11.2 Information provided on the RFLE or by the investigating officer should be used to determine the purpose of the examination. In some cases, evidence may need to be transferred to Trace Evidence for analysis or, depending on the overall case, may not require analysis.

2.11.3 Information such as package labeling, visible precipitates, number of layers present, viscosity and color of liquid should be considered when deciding on an analytical scheme.

2.11.4 The approximate volume of the liquid and pH of aqueous liquids will be determined as sample size allows. The density and solubility of the liquid may be determined.

2.11.5 Additional considerations regarding liquids submitted in association with a clandestine laboratory or the intent to manufacture methamphetamine, methcathinone or amphetamine as per § 18.2-248 J are addressed in the Clandestine Laboratory section.

2.11.6 It may not be sufficient to simply screen a neat liquid with TLC or FTIR. Liquids submitted for general drug analysis may require extraction prior to TLC or GCMS to concentrate the analyte during the screening process or to remove the analyte from the matrix. Screening for suspected amyl nitrite will require GC/MS headspace analysis. Examples of samples which come in liquid forms are:

- Solutions containing GHB, GBL or 1,4-butanediol
- Cough syrups
- Suspected amyl nitrite or other inhalants
- Eye drops or other liquids in dropper bottles
- Injectables such as tubexes

2.12 Plant Material

2.12.1 Initially, all plant material shall be screened via stereomicroscopy for cystolithic hairs.

2.12.1.1 In the presence of cystolithic hairs or marijuana seeds, the analytical scheme for marijuana shall be completed.

2.12.1.2 Contextual clues, such as package labels or banana leaf wrappers, shall be recorded in the case notes.

2.12.1.3 In the absence of cystolithic hairs and in conjunction with contextual clues from the evidence, samples shall be screened for controlled substances including, but not limited to, Salvinorin A, Cathinone/Cathine and Synthetic Cannabinoids.

2.12.1.3.1 Apparent plant material residues, if analyzed, should be handled in the same manner.

2.12.2 Fungal material shall be screened as per the Psilocybin and Psilocyn Methodology (see ¶ 24).

3 DRUG ITEM REDUCTION PROGRAM

3.1 Introduction

3.1.1 The Drug Item Reduction Program (DIRP) allows for the analysis of key items within a case to maximize the resources of the laboratory.

3.1.2 In every case, the most significant items in terms of quantity and schedule are analyzed. This "rule of thumb" cannot address every drug case. Consideration must be given to the information contained on the Request for Laboratory Examination (RFLE). This includes things such as the specific charges or types of offense, items unique to a single suspect, the statement of fact and examinations requested and the descriptions of evidence submitted as well as the chemist's visual inspection of the items.

3.1.3 If, during the pretrial process, it becomes apparent that items not analyzed will require analysis for successful prosecution, then upon re-submission, that item will receive top priority at the laboratory.

3.2 Procedures

3.2.1 Steps should be taken to discourage "no suspect, information only" requests.

3.2.2 Syringes should only be analyzed if they are the only item in the case.

3.2.3 Quantitative analyses will not routinely be performed and only will be done at the request of a Commonwealth's Attorney. A suitable justification should be obtained.

3.2.4 In general, residues in drug paraphernalia, cigarettes or cigarette butts will not be analyzed when measurable quantities of the associated drugs are also included among the items submitted.

3.2.4.1 Example 1: Submitted evidence includes a plastic bag corner containing solid material and a glass tube smoking device with residue. The solid material would be analyzed and the smoking device would not.

3.2.4.2 Example 2: Submitted evidence includes five tablets containing oxycodone and a plastic straw section with residue. The tablets would be analyzed and the straw section would not.

3.2.4.3 Example 3: Submitted evidence includes five tablets containing alprazolam and a plastic straw section with residue. The tablets would be analyzed and the straw section would not unless information on the RFLE indicates that the straw section was used for a different drug.

3.2.4.4 Example 4: Submitted evidence includes a plastic bag of plant material and a glass tube smoking device with residue. Both the plant material and the smoking device would be analyzed.

3.2.5 When multiple residue specimens are submitted within an item (without an item with a measurable quantity), similar residues (e.g., two spoons with residue) may be combined after appropriate screening tests to result in only one GC/MS sample.

3.2.6 Pharmaceutical preparations should be visually examined using pharmaceutical identifiers and appropriate reference compendia.

3.2.6.1 No further analysis is required for misdemeanor offense intact, marked pharmaceutical preparations (e.g., tablets or untampered capsules) indicated as non-controlled or Schedule VI preparations. These may be reported as "Not Analyzed" or using the "Visual examination determined…" language delineated in the Reporting Guidelines section of this manual. See ¶ 7.2.1.4 for tamperable capsules.

3.2.6.2 If identical intact, marked pharmaceutical preparations (e.g., tablets or untampered capsules) are present in multiple items, analysis is required for only one item. Those preparations not analyzed may be reported as "Not Analyzed" or using the "Visual examination determined…" language delineated in the Reporting Guidelines section of this manual. See ¶ 7.2.2.2 for tamperable capsules.

3.2.6.3 Partial pharmaceutical preparations may be not analyzed when intact pharmaceutical preparations or measurable quantities of drugs are present.

3.2.7 If items are not analyzed per this procedure, case notes shall indicate this by a notation of "Not Analyzed" or "DIRP".

4 WEIGHING PRACTICES

4.1 Procedures

4.1.1 Weights for powders, marijuana and other plant materials will be taken prior to sampling.

4.1.2 Dosage units (e.g., cigarettes, cigarette butts, blotter papers, tablets or capsules) will not routinely be weighed.

4.1.3 Analytical, top-loading or high-capacity electronic balances are acceptable for routine casework. The balance used will be recorded in the case notes.

4.1.3.1 Analytical (4 place) balances shall not be used for samples weighing greater than 20 grams.

4.1.4 Weights will be recorded in the analytical notes as they are displayed on the balance.

4.1.4.1 Calculations involving weights will be done using the weights as they are recorded.

4.1.5 Once implemented, the estimated uncertainty will be subtracted from the recorded weight and, subsequently, the last decimal place will be truncated prior to reporting as per ¶ 32.4. Until the Uncertainty of Measurement (UOM) policy is implemented, simply truncate the last decimal place prior to reporting.

4.1.6 If the estimated uncertainty is equal or larger than the weight, a more accurate balance shall be used or the substance shall be reported as a residue, whichever is appropriate.

4.2 Weighing Practices for Administrative Sampling Plan

4.2.1 Simple Possession

4.2.1.1 A gross weight of the material including innermost packaging will be obtained and designated as a gross weight (GW) in the case notes.

4.2.1.1.1 As an alternative, the net weight of the one specimen analyzed will be obtained and designated as a net weight (NW) in the case notes. The gross weight of the remaining specimens including innermost packaging will be obtained, designated as such in the case notes.

4.2.1.2 In cases where the container weight is clearly much greater than the sample weight, obtain the net weight (without packaging) of the material and report appropriately.

4.2.2 Possession with Intent or Distribution

4.2.2.1 Items containing up to five specimens

4.2.2.1.1 The net weight of the total will be obtained and designated as a net weight (NW) in the case notes, except for marijuana where the gross weight is less than ½ ounce.

4.2.2.1.2 In marijuana cases where the gross weight will be less than ½ ounce, the weight may be obtained of the plant material including the innermost packaging and designated as a gross weight (GW) in the case notes.

4.2.2.2 Items containing more than five specimens

4.2.2.2.1 The net weight of the five specimens to be analyzed will be obtained and

designated as such in the case notes, except for marijuana where the total gross weight is less than ½ ounce (see ¶ 4.2.2.2.2 below).

4.2.2.2.1.1 The gross weight of the remaining specimens including innermost packaging will be obtained and designated as such in the case notes.

4.2.2.2.1.2 Both the net weight of the analyzed specimens and the gross weight of the remaining specimens will be reported on the Certificate of Analysis.

4.2.2.2.2 In marijuana cases where the total gross weight is less than ½ ounce, the weight may be obtained of the plant material including the innermost packaging and designated as a gross weight (GW) in the case notes.

4.2.3 Cases with Weight Thresholds

4.2.3.1 In instances where statutory requirements or state sentencing guidelines designate weight thresholds, sufficient specimens will be weighed and analyzed to exceed the threshold. A list of these instances can be found in ¶ 37.

4.2.3.1.1 The net weight of the specimens required to exceed the threshold will be obtained and designated as such in the case notes.

4.2.3.1.2 The gross weight of the remaining specimens including innermost packaging will be obtained and designated as such in the case notes.

4.2.3.1.3 Both the net weight of the analyzed specimens and the gross weight of the remaining specimens will be reported on the Certificate of Analysis.

4.3 Weighing Practices for Hypergeometric Sampling Plan

4.3.1 Resubmissions

The hypergeometric sampling model will be used most often for cases being resubmitted at the request of a Commonwealth's Attorney.

4.3.1.1 Only the net weight of the additional samples will be obtained and reported on the Supplemental Certificate of Analysis.

4.3.2 Initial Submissions

4.3.2.1 The net weight of each specimen requiring analysis will be obtained and designated as such in the case notes.

4.3.2.2 The gross weight of the remaining specimens including innermost packaging will be obtained and designated as such in the case notes.

4.3.2.3 Both the net weight of the analyzed specimens and the gross weight of the remaining specimens will be reported on the Certificate of Analysis.

5 SAMPLING

5.1 Introduction

Sampling evidence is the most important initial step in forensic drug analysis. One must be sure that what is sampled is truly representative of the total population. The analyst must take into consideration the homogeneity (or lack thereof) among drug packaging (bags, packets, capsules, etc.) and its contents. Careful visual inspections and personal experience are essential in determining the proper sampling procedure.

For items containing multiple specimens, statistically-based sampling models (e.g., hypergeometric distribution) will allow the analyst to analyze a portion of the specimens and subsequently make statistical inferences about the population. Alternatively, a fixed number of specimens within a population may be analyzed with the purpose in mind of meeting the requirements of a particular criminal charge (e.g., simple possession, distribution). In these instances, an inference to the entire population will not be drawn and the number of specimens that were analyzed will be indicated on the Certificate of Analysis.

5.2 General Sampling

5.2.1 Every effort should be made to avoid handling evidence repeatedly. The material should be sampled and immediately sealed. If necessary, the evidence may be closed and maintained in short term storage until the analysis is complete. Evidence generally will not remain in short term storage for longer than 30 days.

5.2.2 In order to minimize detailed labeling on small items such as very small metal foil packets, plastic bags or plastic bag corners, they may be secured in a bandolier of tape, which is then labeled. If needed, items may be placed in an additional plastic bag which can be sealed, fully labeled and properly documented in the case notes.

5.2.3 For chemical analyses, a representative sample shall be removed from the specimen. When sample size allows, testing should be applied on separate samplings of the material. Taking a small amount of material for use in a color test prior to taking a separate sampling for additional tests is an appropriate method. For suspected marijuana, performing the microscopic examination on a larger population prior to taking a representative sample for thin layer chromatography and the Duquenois-Levine test will suffice. For pharmaceutical tablets and capsules, the use of pharmaceutical identifiers as a screening test prior to taking a representative sample for confirmatory testing will suffice.

5.3 Administrative Sampling Plan

The administrative sampling plan will be used in cases to answer a specific legal question. If more specimens than listed below need to be analyzed for successful prosecution, additional analysis utilizing the hypergeometric sampling plan will be conducted upon written request from the Commonwealth's Attorney. Items requiring quantitative analysis should be analyzed using the hypergeometric sampling plan.

5.3.1 Simple possession

5.3.1.1 One specimen will be randomly selected and fully analyzed.

5.3.1.2 All remaining specimens will be left intact in case further analysis is required.

5.3.2 Possession with intent to distribute or distribution

5.3.2.1 Items containing up to 5 specimens

Each specimen will be analyzed separately and fully.

5.3.2.2 Items containing greater than 5 specimens

5.3.2.2.1 Five randomly selected specimens will be analyzed separately and fully.

5.3.2.2.2 The remaining specimens will be left intact in case further analysis is required.

5.3.3 Cases with weight thresholds

5.3.3.1 In instances where statutory or state sentencing guidelines have weight thresholds, enough specimens will be weighed and analyzed, separately and fully, to exceed the threshold. A list of these instances can be found in ¶ 38.

5.3.3.2 The remaining specimens will be left intact in case further analysis is required.

5.3.4 Pharmaceutical preparations

5.3.4.1 Due to the unique physical identifiers present in pharmaceutical preparations, a consistent sample population can easily be determined. The thoroughness represented by the sampling scheme used for street drugs is not required for pharmaceutical preparations which are clearly visually consistent with each other.

5.3.4.2 For drug substances involving misdemeanor prosecutions in Schedules V and VI, sampling is not normally required. For drug substances involving Schedule IV and above, at least one representative sample must be analyzed fully.

5.3.4.2.1 For tamperable dosage units, screen a sample chosen using the hypergeometric scheme described below utilizing TLC and/or color tests prior to fully analyzing one unit.

5.3.4.2.1.1 If tampering is suspected, analyze dosage units utilizing the hypergeometric scheme.

5.3.4.2.2 If the evidence is resubmitted for further analysis, resample and analyze using either the administrative sampling plan (¶ 5.3.2) or the hypergeometric sampling scheme (¶ 5.4) depending on the legal requirements.

5.3.5 Exceptions to this Plan may occur only at the discretion of the Section Supervisors in consultation with the Chemistry Program Manager.

5.4 Hypergeometric Sampling Plan

5.4.1 Hypergeometric sampling is a statistically-based model involving a defined confidence level with an associated probability of finding failures in a population. (¶¶ 5.9.1, 5.9.2) The hypergeometric model is used for specimens with no significant markings or labels (e.g., the contents of plastic bags and bag corners, vials, and glassine packets). This model should also be used when the item requires a quantitative analysis.

5.4.1.1 Hypergeometric sampling may be used when additional analysis is requested for successful prosecution.

5.4.1.2 The appropriate number of specimens within the population will be randomly selected to give a 95% confidence level that at least 90% of the population contains the analyte of question. Refer to ¶ 39 of this manual.

5.4.1.3 Record the number of specimens indicated by the table in ¶ 39 along with an indication of the statistical relevance of the number in the case notes.

5.4.1.4 Each specimen sampled will be analyzed separately and fully.

5.5 Multiple Specimens

5.5.1 If all specimens are not analyzed, the number of those that are fully analyzed will be recorded in the case notes.

5.5.2 Weights and autosampler vial numbers will be associated with specific specimens by the use of sub-numbering in the case notes.

5.5.3 Within any sampling scheme, Administrative or Hypergeometric, if the first set of observations determines that more than one population is present, further samples from each population must be taken.

5.5.4 If presumptive testing indicates that no controlled substances are present in the samples chosen, a screening test must be done using the hypergeometric sampling scheme.

5.5.4.1 For items consisting of specimens, which are obviously non-controlled such as gum, candy or vitamins, a single representative sample may be screened.

5.6 Bulk Materials

Bulk materials (e.g., bricks of compressed powder, bales of plant material) should be broken or cored to obtain a representative sample. Depending on the size of the material, samples from several locations may be required to obtain a representative sample. The examiner will record the locations from which the samples were obtained in the case notes.

5.7 Residue Samples

Residues are samples which are either too small to be weighed accurately or that which remains after the bulk has been removed. Residues can be sampled by mechanical means (e.g., shaking or scooping) or chemical means (e.g., rinsing with solvent). Case notes must reflect the method by which the sample was removed.

5.7.1 When possible, a sample should be removed while leaving a portion of the residue intact.

5.7.2 When it is not possible to redeposit and return the residue as received, the extract used in analysis will be returned to the evidence as per the Quality Manual (¶ 14.10.5).

5.7.2.1 Procedure: Evaporate the solvent from the extract in the autosampler vial used in analysis. Seal the autosampler vial (ASV) into a ziplock bag. Label the ziplock bag with the FS Lab #, Item #, initials and a statement similar to "vial and bag added at lab." Record the date in the case notes that the ASV was placed in the evidence.

5.8 Sampling for Quantitative Analysis

5.8.1 Quantitative analyses require homogenized representative samples. Generally, a relatively large sample is homogenized with a mortar and pestle prior to taking the small samples required by the quantitative method to make the solutions. The remainder of the homogenized portion should be returned with the evidence in a suitably labeled plastic bag provided by the laboratory. By their nature, hash oil samples should require no further homogenization.

5.8.2 Single specimen items

5.8.2.1 If the specimen is approximately 1 gram or less, homogenize the entire specimen, take the two samples required for the quantitation method and return the bulk of the material to the evidence.

5.8.2.2 If the specimen is more than 1 gram, take an approximately 1 gram portion to homogenize. Take the two samples required for the quantitation method and return the homogenized portion to the evidence.

5.8.2.2.1 For large specimens such as kilos of cocaine, either a core sample or combined samples taken from multiple locations should be used for the homogenizing process. The locations of samples taken shall be described in the case notes.

5.8.3 Multiple specimen items

5.8.3.1 Items with multiple specimens should be analyzed qualitatively using the hypergeometric sampling plan.

5.8.3.2 A composite will be formed consisting of portions from each of the specimens analyzed in the hypergeometric sampling plan. If sample size allows, the composite should consist of at least 1 gram but not more than 5 grams of material. Homogenize the composite and take the two samples required for the quantitation method. The remainder of the composite should be returned to the evidence in a ziplock bag provided by the laboratory, clearly marked as a composite.

5.9 References

5.9.1 Shark, Robert E. "Sampling Your Drugs: A User's Guide", Commonwealth of Virginia, Bureau of Forensic Science, Technical Brief, c. 1986.

5.9.2 Frank, Richard S. *et. al.* "Representative Sampling of Drug Seizures in Multiple Containers." *Journal of Forensic Sciences*, JFSCA, Vol. 36, No. 2, March 1991, pp. 350-357.

5.9.3 Colon, Maria *et.al.* "Representative Sampling of 'Street' Drug Exhibits" *Journal of Forensic Sciences*, JFSCA, Vol. 38, No.3, May 1993, pp. 641-648.

5.9.4 SWGDRUG Recommendations, 5.1 ed. "PART III A - Methods of Analysis/Sampling Seized Drugs for Qualitative Analysis", January, 2011.

5.9.5 European Network of Forensic Science Institutes Drugs Working Group, *Guidelines on Representative Drug Sampling*, 2003.

6 MARIJUANA AND HASHISH OIL

6.1 Introduction

6.1.1 Marijuana is neither a "controlled substance" nor is it "scheduled" under Virginia Law. However, it is defined and covered under separate sections of the Drug Control Act of Virginia and has associated penalties.

6.1.2 Cannabis (marijuana) contains tetrahydrocannabinol (THC) in both the male and female plants. THC is found in all parts of the plant in varying concentrations.

6.1.3 Hashish oil is an oily extract containing one or more cannabinoids with little, if any, plant material present and containing 12% or more THC. It is controlled in Schedule I.

6.2 Macroscopic Identification

6.2.1 Gross morphological characteristics that may be observed include the palmate arrangement of the leaflets, the pinnate appearance of the leaflets, the serrated edges of the leaflet, the buds (with or without seeds) and, if present, fluted stems and stalks.

6.2.2 Due to the compressed or mutilated nature of many samples, many of these characteristics may not be discernable.

6.2.3 Positive macroscopic examination results may be recorded in the analytical notes by the use of an abbreviation of positive for characteristics of Marijuana (e.g., pos. characteristics MJ), a plus (+), or a plus circled (⊕). A result is considered positive when sufficient characteristics are observed and are specified in the case notes. Negative observations may be recorded in a similar fashion.

6.3 Microscopic Identification

6.3.1 View the sample at varying magnifications (approximately 10 – 40x) using a stereomicroscope.

6.3.2 Cystolithic hairs are unicellular, “bear claw” shaped hairs with a cystolith of $CaCO_3$ at the base. They are found in greatest abundance on the upper side of the leaf with longer covering hairs on the underside.

6.3.3 Seeds are coconut shaped, veined (with lacy markings) and have a ridge around the circumference.

6.3.4 The observation of the presence of appropriate cystolithic hairs or characteristic seeds is sufficient for a positive test. The observation of additional characteristics is considered supportive. Positive microscopic examination results will be recorded in the analytical notes by the use of an abbreviation of positive for characteristics of Marijuana (e.g., pos. characteristics MJ), a plus (+), or a plus circled (⊕). A result is considered positive when sufficient characteristics are observed. Negative observations will be recorded in a similar fashion.

6.4 Thin Layer Chromatography (TLC)

6.4.1 TLC plates should be silica gel or equivalent, sufficient to resolve the three major cannabinoids listed in ¶ 6.4.6.4 (examples: Analtech Silica Gel GHLF 250um 10 X 20, Analtech Silica Gel 250um GF 10x20, EMD TLC Silica Gel 250um 60 F_{254} 5 X 10, EMD TLC Silica Gel 250um 60 F_{254} 10 X 20).

6.4.2 Extract sample into a suitable solvent (e.g., hexane, petroleum ether or methanol). The solvent used must be recorded in the case notes.

6.4.3 Spot sample(s), standard(s) and solvent blank on the plate. The maximum number of spots when using a 10 x 20 cm plate is 32. (See ¶ 9.3 for further information)

6.4.4 The results of the blank must be recorded in the case notes. This may be done by using a check mark (√) or "ok" or "-" to record that the results of the blank were acceptable (e.g., Blk √).

6.4.5 Mobile Phase: 4-8% diethylamine in toluene

6.4.6 Visualization Sprays

6.4.6.1 Fast Blue B Salt (Tetrazotized o-dianisidine zinc chloride salt)

6.4.6.2 Fast Blue BB Salt (4-benzoylamino-2,5-diethoxy-benzenediazonium chloride hemi [zinc chloride] salt)

6.4.6.3 Reagent preparation is listed in the Thin Layer Chromatography section of this manual. (see ¶ 9.5.6)

6.4.6.4 Results:

- The three major cannabinoids migrate and develop in the following order:
  - Top spot - Cannabidiol – orange
  - Middle spot - Tetrahydrocannabinol (Δ^9-THC) - red
  - Lower spot - Cannabinol – purple
- A red spot at the origin may be present in unburned marijuana due to cannabinolic acid(s).

6.4.7 Samples will be screened for the presence of other commonly encountered drugs such as cocaine or PCP by either overspraying with acidified iodoplatinate or by running a separate plate in an appropriate drug bath (e.g., TLC1 or TLC2.) (see ¶ 9.4)

6.4.8 Specific solvent systems and developing sprays utilized in casework will be denoted in the analytical case notes. Positive TLC results may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) along with the standard used in the comparison. A result is considered positive when the distance traveled and the reaction with the visualization methods compare favorably with a standard. It is not necessary for each of the three major cannabinoids to be present for the results to be considered positive. Negative reactions may be recorded in a similar fashion.

6.4.9 After the plate is sprayed with FBB or FBBB, it shall be scanned and a color hardcopy printed. Each lane shall be labeled (FS Lab# and Item #, standard identifier, blank, etc.) either in the image or on the original color copy. Templates may be used to assist with labeling.

6.4.9.1 When multiple samples/cases are run on the same plate, the color printout shall be stored in one case file.

6.4.9.2 Black and white copies of the original shall be placed in other case files with a reference to where the original is stored.

6.5 Duquenois-Levine

6.5.1 Extract sample into a suitable solvent (e.g., hexane, petroleum ether or methanol). If a large amount of solvent is used, most of it must be evaporated.

6.5.2 Add approximately equal amounts of Duquenois reagent and concentrated HCl to extract. A positive reaction to the Duquenois portion is a blue/purple color.

6.5.3 Add sufficient $CHCl_3$ to form two discernable layers and mix. For a positive reaction to the Levine portion of the test, the bottom layer turns pink/purple in the presence of THC or other cannabinoids.

6.5.4 Run a solvent blank as a negative control with each batch of samples. The results of the negative control must be documented in the case notes. This may be done by using a check mark (√) or "ok" or "-" to record that the results of the blank were acceptable (e.g., Blk √).

6.5.4.1 If a color develops in the blank, it should be repeated to determine the source of the contamination.

6.5.4.1.1 If the results of the second blank are acceptable, all samples should be re-run.

6.5.4.1.2 If the results of the second blank are unacceptable or if the blank and samples are not available to be re-tested, the analyst should take steps to resolve the issue (e.g., replacing the solvent in the bottle, checking the reagents) prior to re-sampling and any further analysis.

6.5.5 Reagent preparation is listed in the Color Test section of this manual. (see ¶ 8.3.6)

6.5.6 The Rapid Duquenois-Levine Procedure

6.5.6.1 Place a small amount of plant material in a culture tube, add Duquenois reagent and concentrated HCl in approximately equal proportions. Observe a blue/purple color. Add $CHCl_3$ and observe extraction of pink/purple color into the $CHCl_3$ layer.

6.5.6.2 A blank (negative control) will be run in a separate culture tube. The results of the negative control must be documented in the case notes.

6.5.6.2.1 If a color develops in the blank, it should be repeated to determine the source of the contamination.

6.5.6.2.2 If the results of the second blank are acceptable, all samples should be re-run.

6.5.6.2.3 If the results of the second blank are unacceptable or if the blank and samples are not available to be re-tested, the analyst should take steps to resolve the issue (e.g., replacing the solvent in the bottle, checking the reagents) prior to re-sampling and any further analysis.

6.5.6.3 If the Rapid Duquenois-Levine is utilized, it should be recorded in the case notes.

6.5.7 Results must be recorded in the case notes. This may be accomplished either with a single plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) indicating a positive result in both steps or the colors of each step may be noted. Negative reactions may be recorded in a similar fashion.

6.6 Gas Chromatography/Mass Spectrometry (GC/MS)

6.6.1 GC/MS shall be performed if the results from any of the prior three tests are inconclusive.

6.6.2 Retention time data is not required.

6.7 Hashish Oil

6.7.1 Analytical Scheme

6.7.1.1 Weigh or approximate the volume of the material.

6.7.1.2 Remove a representative sample for testing.

6.7.1.3 Dilute with appropriate solvent and perform TLC as listed for marijuana. The presence of additional cannabinoids will confirm that the THC is most likely from the marijuana plant.

6.7.1.4 Perform the Duquenois-Levine test.

6.7.1.5 Dilute with appropriate solvent and run on GC/MS to confirm the presence of THC.

6.7.1.6 Quantitate THC using method below.

6.7.2 THC Quantitation

6.7.2.1 Materials

- n-Hexane: High purity
- Ethyl Alcohol: (95%) USP Grade
- Delta-9-Tetrahydrocannabinol: (10 mg/mL in EtOH)
- n-Triacontane: 99% pure or greater.
- 4N NaOH
- Class A volumetric pipettes
- Analytical balance

6.7.2.2 Internal Standard Solution

6.7.2.2.1 Prepare a sufficient volume to dilute the THC standard solution and all samples.

6.7.2.2.2 Prepare a 1 mg/mL solution of n-triacontane in n-hexane in the appropriate volumetric flask.

6.7.2.2.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

6.7.2.3 THC Standard Solution

6.7.2.3.1 Quantitatively transfer the contents of the ampoule (1 mL of ethanol containing 10 mg THC) to a 10 mL volumetric flask using 95% EtOH. Dilute to volume with 95% EtOH and mix thoroughly. This results in a 1 mg/mL stock solution.

6.7.2.3.2 Pipette 2 mL of the stock solution into a culture tube. Evaporate to dryness. Pipette 2 mL of internal standard solution into the culture tube and mix thoroughly. This results in a 1.0 mg/mL solution of THC in internal standard solution.

6.7.2.3.3 Prepare a solution of another concentration within the linear range to use as the check standard. Using a volumetric pipette, transfer 5 mL of the stock solution into a test tube. Evaporate to dryness. Pipette 2 mL of internal standard solution into the culture tube and mix thoroughly. This results in a 2.5 mg/mL solution of THC in internal standard solution.

6.7.2.4 Sample Preparation

Prepare two separate sample solutions. For each, weigh 10-20 mg of sample into a test tube and add 5 mL internal standard solution via pipette.

6.7.2.5 GC parameters

- Column 15 m HP-1 (0.25 mm i.d., 0.25 μm film thickness)
- Oven temperature: approximately 240-260 °C

6.7.2.6 THC acid converts to THC upon heating in the injection port, causing high quantitative results if present. To avoid this, after quantitation, transfer the contents of the autosampler vial to a test tube. Add 1 mL 4N NaOH solution to the test tube, shake vigorously for 30 seconds and let settle. Pipette the hexane into a new autosampler vial and re-inject. If the values are approximately the same, no acid was present. Large differences would be expected if a significant red spot at the origin was noted during the TLC test.

6.7.2.6.1 If the results are less than 10% different, report the initial values.

6.7.2.6.2 If the results are equal or more than 10% different, extract the standard and each sample with base and requantitate.

6.7.2.7 Linear Range

6.7.2.7.1 The validated linear range of the THC method is 0.5 – 5 mg/mL.

6.7.2.7.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

6.7.2.8 THC elutes before n-triacontane.

6.7.2.9 THC solutions and internal standard solutions should be closed and stored in the refrigerator when not in use.

6.7.2.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 10.4.4.7 – 10.4.4.10

6.7.3 Reporting

6.7.3.1 Hashish oil will be reported as currently defined by the Code of Virginia. Current example: Hashish oil (Schedule I), which contains ___% tetrahydrocannabinol by weight.

6.8 Extraction of THC from Food Products (candy, brownies, etc.)

6.8.1 If plant material is visible, remove sample of plant material and analyze appropriately. If an extraction is necessary, see ¶ 6.8.2.

6.8.2 Extraction of THC from Food Products (6.9.3)

A procedure blank shall be run with the extraction and documented in the case notes.

- Add hexane to suitable quantity of sample.
- Vortex and Centrifuge.
- Transfer hexane to a new test tube.

- Extract with 0.5N KOH (methanolic solution). The bottom layer retains THC if present.
- Discard top hexane layer.
- Wash with at least 3 aliquots of hexane.
- Acidify using 1N HCl to pH 1-2.
- Extract with hexane (top layer, retains THC).
- Dry hexane extract with sodium sulfate.
- Remove and retain hexane.
- Concentrate hexane through evaporation.
- Resultant concentrated extract will yield THC.

6.9 References

6.9.1 C.G Pitt, R.W. Hendron and R.S. Hsia, "The Specificity of the Duquenois Color Test for Marijuana and Hashish", *Journal of Forensic Sciences*, 1972, Volume 17, No. 4, Pages 693-700.

6.9.2 G.R. Nakamura, " Forensic Aspects of Cystolithic Hairs of Cannabis and Other Plants", *Journal of the Association of Official Analytical Chemists,* Volume 52, No. 1, 1969, Pages 5- 16.

6.9.3 Ely, Roger, CLIC List Communication, 2007.

7 PHARMACEUTICAL IDENTIFIERS

7.1 Introduction

Pharmaceutical preparations possess unique identifying information both in the general appearance of the preparation and the inscriptions or markings.

7.2 Procedure

7.2.1 It is normally acceptable to visually examine intact, marked tablets or untampered, marked capsules in those cases involving misdemeanor prosecutions in Schedules V and VI. Results should be reported as given in the Reporting Guidelines section of this manual. (See ¶ 33.6)

7.2.1.1 Tablet descriptions in case notes should clearly reflect the physical characteristics used in the visual examination.

7.2.1.2 Check the PDR, Poison Control, DEA Logo Index, Identadrug, Drug ID Bible or other similar sources for information relating to inscriptions on tablets and capsules. Two unrelated references are recommended for unfamiliar tablets.

7.2.1.2.1 The physical characteristics, such as shape and color, should be considered in addition to the comparison of the markings. Tablets with partial markings may be reported as "visually examined" if they are mixed with intact tablets which are identical in other aspects. If the partial markings are not clear enough to compare to the intact tablets, a screening test should be performed.

7.2.1.2.2 Reference information including page number or a hardcopy of results from an electronic database shall be recorded in the case notes.

7.2.1.3 It should be recorded in the case notes if any tampering is evident from the dosage unit appearance.

7.2.1.4 Tamperable capsules should be screened for tampering using appropriate color tests or TLC using the hypergeometric sampling scheme.

7.2.1.5 If tampering is not detected, it may be acceptable to report as visually examined.

7.2.1.6 If tampering is suspected, then a complete analytical scheme including a structural elucidation technique is required for identification.

7.2.2 At least one dosage unit must be fully tested in those cases involving Schedule IV and above.

7.2.2.1 Visually examine the tablets, capsules, etc. to determine that their size, color and markings are consistent. Check the PDR, Poison Control, DEA Logo Index, Identadrug, Drug ID Bible or other similar sources for information relating to inscriptions on tablets and capsules. Only one reference is necessary. Reference information including page number or a hardcopy of results from an electronic database should be recorded in the case notes.

7.2.2.2 Tamperable capsules should be screened for tampering using appropriate color tests or TLC using the hypergeometric sampling scheme.

7.2.2.3 If all dosage units are visually similar and if tamperable capsules have consistent screening results, take one representative sample for analysis.

7.2.2.4 A structural elucidation technique must be used within the analytical scheme.

7.2.2.4.1 If the results of the analysis are consistent with the manufacturer's specifications with regard to content, the results shall be reported as outlined in the Reporting Guidelines, ¶ 33.7.

7.2.2.4.2 If the results of the analysis are inconsistent with the manufacturer's specifications with regard to content, further analysis may be required.

7.2.2.4.2.1 For items with one specimen, the results shall be reported as outlined in the Reporting Guidelines, ¶ 33.8.1.

7.2.2.4.2.2 For items with multiple specimens, hypergeometrically screen using appropriate color tests, TLC or AccuTOF-DART. If screening indicates that specimens are consistent with each other, continue to ¶ 7.2.2.4.2.2.1. If screening indicates that specimens are inconsistent, further analysis to characterize populations will be necessary.

7.2.2.4.2.2.1 One dosage unit from each population shall be fully analyzed and reported for charges of simple possession. (See ¶ 33.8.1)

7.2.2.4.2.2.2 Five dosage units from each population shall be fully analyzed and reported for charges of possession with intent to distribute or distribution. (See ¶ 33.8.2)

7.2.3 When the sample is not an identifiable pharmaceutical preparation, it is required that a definitive structural elucidation technique be used within the analytical scheme, if the substance is to be reported.

7.2.4 Physical identifiers serve as an effective preliminary test within the full analytical scheme.

7.2.5 "No controlled substances found" or "No controlled substances identified" may be used for reporting those non-controlled substances not structurally identified.

8 COLOR TESTS

8.1 Introduction

8.1.1 Color tests are used as a screening test at the beginning of an analysis. Most are performed on clean porcelain or disposable plastic spot plates; however some may be performed in disposable culture tubes (e.g., Scott's, Tannic Acid).

8.1.2 Thin Layer Chromatography visualization sprays may act as color tests when sprayed on a TLC plate or filter paper where a drop of sample solution has been placed.

8.2 Procedures

8.2.1 The test reagent should be added to the plate or tube first, and then the questioned sample. This practice determines if the plate or tube was clean before the analysis.

8.2.1.1 If a reaction occurs prior to the addition of the sample, the plate or tube shall be discarded or cleaned before testing the sample.

8.2.2 Several of the listed reagents have more than one recipe listed. Any of the listed, referenced recipes may be utilized in casework and should be reflected in the reagent logbook.

8.2.3 The Department *Reagent Worksheet* shall be used to record reagent preparation.

8.2.3.1 It is acceptable to make final volumes different than those listed below as long as the amount of each component is recorded.

8.2.4 Reagents should be made in quantities to minimize waste. The shelf life of color test reagents is two years, unless otherwise listed.

8.2.5 Reagents, indicators and solutions listed in the USP-NF may be used for their published purposes.

8.2.5.1 Positive controls and blanks shall be performed when using reagents or tests listed in the USP-NF. The results shall be recorded in the case notes.

8.2.5.2 Case notes shall include the procedure with the appropriate reference and the results of the test.

8.2.6 Positive color reactions are noted in each of the individual drug sections. These positive reactions may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos). The color observed must be noted for drugs not routinely encountered. Negative reactions may be recorded in a similar fashion. (For Duquenois-Levine results see ¶¶ 6.5.2 and 6.5.3)

8.2.7 Most color test reagents are comprised of strong acids and chemicals requiring careful handling. Appropriate safety precautions should be observed. Refer to MSDSs for storage and handling.

8.3 Color Tests and Reagents

The following lists the commonly used color test reagents and some examples of reactions with various drugs. The references for each test are in parenthesis.

8.3.1 Bates Test (¶ 8.4.5) tests for cocaine base.

8.3.1.1 Procedure: The Bates test is used as the second part of the Cobalt thiocyanate test (¶ 8.3.4). If the Cobalt thiocyanate test is negative, add Marquis reagent to spot well.

8.3.1.2 Results: The formation of a very blue precipitate indicates cocaine base, other compounds give weaker blue or no reaction.

8.3.2 Benedict's Solution (¶ 8.4.2) tests for reducing sugars and some antibiotics.

8.3.2.1 Recipe: 1.73 g of copper sulfate in 10 mL of water. With the aid of heat, dissolve 17.3 g trisodium citrate and 10 g of anhydrous sodium carbonate in 80 mL of H_2O. Pour the two solutions together and let cool. Dilute to 100 mL with water.

8.3.2.2 Procedure: Add 0.5 mL of the reagent to sample and heat.

8.3.2.3 Results:

- Ascorbic acid, strong reducing agents, glucose, tetracycline – red
- Streptomycin - orange/brown

8.3.3 Chen's Test (¶ 8.4.2) tests for phenethylamines.

8.3.3.1 Recipe: 1 g copper sulfate and 1 mL glacial acetic acid in 100 mL H_2O.

8.3.3.2 Procedure: Make an approximate 1% aqueous solution of the sample, add equal volumes of Chen's reagent and 2N NaOH.

8.3.3.3 Results: ephedrine, PPA and pseudoephedrine – purple

8.3.4 Cobalt Thiocyanate reacts with tertiary and quaternary amines to form a blue precipitate and is used for general screening. May be used in conjunction with the Bates test (¶ 8.3.1) or the Stannous Chloride test (¶ 8.3.21).

8.3.4.1 Recipes:

- 2 g cobalt thiocyanate in 100 mL H_2O or methanol (¶ 8.4.1)
- 2 g cobalt thiocyanate in 100 mL H_2O and 100 mL of glycerine. (¶ 8.4.3)
- 1.4 g $CoCl_2$. $6H_2O$ and 0.9 g NH_4SCN in 100 mL H_2O. (¶ 8.4.7)

8.3.4.2 Procedure: Place reagent in well and add sample.

8.3.4.3 Results:

- Cocaine HCl – blue precipitate forms, cocaine base may be initially negative or faintly blue, but blue intensifies upon the addition of dilute HCl.
- PCP - blue
- Amitriptyline / doxepin - blue
- barbiturates with unsaturated side chain (i.e., butalbital) - faint blue

8.3.5 Dille - Koppanyi Test (¶ 8.4.9) reacts with barbiturates.

8.3.5.1 Recipe:

- DK1: 0.1 g cobaltous acetate tetrahydrate in 100 mL methanol plus 0.2 mL glacial acetic acid
- DK2: 5 mL isopropyl amine in 95 mL methanol

8.3.5.2 Procedure: This is a two part test. Place 2 drops of DK1 reagent in a well. Add sample. Add 1 drop of DK2 reagent. When doing multiple samples, they should be separated to avoid cross-contamination due to reagent spreading.

8.3.5.3 Results:

- barbiturates - blue purple
- theophylline, glutethimide and hydantoins - purple
- ampicillin - brown

8.3.6 Duquenois - Levine Test (¶¶ 8.4.3, 8.4.4) reacts with marijuana and hash oil.

8.3.6.1 Recipe: 4 g vanillin and 2.5 mL fresh acetaldehyde per 200 mL 95% ethanol

8.3.6.2 Procedure: See Marijuana section (¶ 6.5).

8.3.6.3 Results: marijuana/hash oil – blue/purple, pink/purple extracts into $CHCl_3$

8.3.7 Ehrlich's Reagent (¶ 8.4.7) reacts with indole moiety and some amines.

8.3.7.1 Recipe: 5 g p-dimethylaminobenzaldehyde to 50 mL of 95% ethanol and 50 mL of conc. HCl

8.3.7.2 Procedure: Place reagent in well and add sample.

8.3.7.3 Results:

- LSD, psilocyn - purple (beware of leaching of dyes in blotter paper or tablets)
- benzocaine, procaine - yellow

8.3.8 Fehlings Solution (¶ 8.4.8) reacts with reducing compounds such as sugars.

8.3.8.1 Recipe:

- Fehlings1 - 3.46 g copper sulfate per 50 mL H_2O
- Fehlings2 - 86.5 g sodium potassium tartrate and 35 g of NaOH per 250 mL of H_2O

8.3.8.2 Procedure: Dissolve sample in water and mix. Add 5 drops of Fehlings1 and 5 drops of Fehlings2 and mix. Heat on steambath for approximately 5 minutes or until warm.

8.3.8.3 Results: reducing sugars – yellow to red.

8.3.9 Ferric Chloride ($FeCl_3$) tests for phenols and GHB.

8.3.9.1 Recipes:

- 9% aqueous solution (¶ 8.4.14)
- 5% aqueous solution (¶ 8.4.2)

8.3.9.2 Procedure: Place sample into a solution of water or methanol and add a drop of reagent.

8.3.9.3 Results:

- salicylamide - dark purple
- acetaminophen - blue
- hydrolyzed aspirin – purple (to hydrolyze a sample, place in H_2O, add a little acid and heat)
- GHB – red/brown

8.3.10 Fiegel's / Nitroprusside (nitroferricyanide) (¶¶ 8.4.3, 8.4.8) for secondary amines.

8.3.10.1 Recipe: 1 g of sodium nitroprusside in 100 mL H_2O and 10 mL acetaldehyde

8.3.10.2 Procedure: Dissolve sample in 2N Na_2CO_3 and add reagent.

8.3.10.3 Storage: store in brown bottle and refrigerate.

8.3.10.4 Results: secondary amines - deep blue color

8.3.11 Froehde's (¶¶ 8.4.1, 8.4.2) reacts with narcotics and is used for general screening.

8.3.11.1 Recipe: 0.5 g ammonium molybdate per 100 mL H_2SO_4 (conc.)

8.3.11.2 Procedure: Place reagent in well and add sample.

8.3.11.3 Results:

- heroin - purple → green
- codeine - green → red/brown
- morphine - deep purple → slate

- aspirin - blue → purple
- phenoxymethylpenicillin - blue
- pentazocine – blue
- acetaminophen – pale blue

8.3.12 GHB Color Test #3 (Smith Test) (¶ 8.4.13) for GHB powders and solutions. This test will not react with GBL or 1,4-butanediol.

8.3.12.1 Recipe:

- Bromocresol Green – 0.03 g bromocresol green in 100 mL of 4:1 methanol:DI water adjusting the pH to 7.0 with 0.1N sodium hydroxide
- Methyl Orange – 0.01 g methyl orange in 100 mL DI water adjusting the pH to 7.0 with 0.1 N sodium hydroxide
- Modified Schweppes Reagent: Mix solutions A and B
  - Solution A – 2 g dextrose in 20 mL of DI water
  - Solution B – 2.4 g aniline hydrochloride in 20mL methanol
- Mix Bromocresol Green and Methyl Orange together in a 1:1 ratio. Then, mix that indicator mixture with the modified Schweppes reagent in a 3:1 ratio.

8.3.12.2 Procedure: Add 0.5 mL of a liquid sample or a small amount of powder to a test tube. Add 2 drops of the mixed reagent and gently swirl.

8.3.12.3 Results:

- GHB – immediate green color
- Negative results – pinkish orange (generally the same or slightly darker than the original test solution)

8.3.13 Marquis (¶¶ 8.4.1, 8.4.2) reacts with opiates and phenethylamines and is used for general screening.

8.3.13.1 Recipe:

- 10 mL 37% formaldehyde in 90 mL H_2SO_4 (conc.)
- 2 mL 37% formaldehyde in 75 mL H_2SO_4 (conc.)

8.3.13.2 Procedure: Place reagent in well and add sample.

8.3.13.3 Storage: Keep tightly capped.

8.3.13.4 Results:

- opiates - purple
- amphetamine/methamphetamine - orange/brown
- aspirin – pink → deep red on standing
- phenoxymethylpenicillin - red
- MDA/MDMA - black

8.3.14 Mayer's Reagent (¶ 8.4.7) reacts with alkaloids

8.3.14.1 Recipe: Dissolve 1 g of mercuric chloride in 100 mL H_2O. Add solid potassium iodide until the reddish precipitate first formed dissolves. Reagent should be clear and pale yellow in color.

8.3.14.2 Procedure: Add 0.1 N HCl to a test tube. Add sample to acid and mix. Add Mayer's reagent to the acid solution.

8.3.14.3 Results: alkaloids – a white to yellow precipitate is formed

8.3.15 Mecke's (¶¶ 8.4.1, 8.4.2) reacts with narcotics and is used for general screening.

8.3.15.1 Recipe: 1 g selenious acid per 100 mL H_2SO_4 (conc.)

8.3.15.2 Procedure: Place reagent in well and add sample.

8.3.15.3 Results:

- heroin - green/blue
- codeine - bright-green/blue green
- PCP - light yellow
- quinine - light yellow

8.3.16 Methylene Blue (¶ 8.4.10) reacts with vitamin C.

8.3.16.1 Recipe: 12.5 mg of methylene blue dissolved in 25 mL of 95% ethanol.

8.3.16.2 Procedure: Add reagent to well and add sample. It may be helpful to run a blank to compare the results.

8.3.16.3 Results: Vitamin C - slowly decolorizes solution from dark blue to light blue.

8.3.17 Nitric Acid (HNO_3) (¶¶ 8.4.1, 8.4.2) reacts with opiates and mescaline.

8.3.17.1 Recipe: concentrated nitric acid

8.3.17.2 Procedure: Place reagent in well and add sample.

8.3.17.3 Results:

- heroin - yellow green
- morphine - red
- codeine - orange

- mescaline - red
- acetaminophen – fumes, orange brown

8.3.18 Parri (¶ 8.4.11) reacts with barbiturates.

8.3.18.1 Recipe: cobaltous acetate (solid), barium oxide (solid), and methanol

8.3.18.2 Procedure: Mix cobaltous acetate, BaO and powdered sample in equal parts in a spot plate well, add methanol.

8.3.18.3 Results: barbiturates – blue

8.3.19 Scotts - Modified Cobalt Thiocyanate (¶ 8.4.3) reacts with cocaine.

8.3.19.1 Recipe: 2 g cobalt thiocyanate in 100 mL H_2O and 100 mL of glycerine.

8.3.19.2 Procedure: Add reagent to well or tube and add sample. Dissolve the blue precipitate from the $Co(SCN)_2$ by the addition of HCl. Add $CHCl_3$

8.3.19.3 Results: cocaine - blue color in the lower ($CHCl_3$) layer.

8.3.20 Silver Nitrate (¶ 8.4.15) indicates the presence of chloride ions.

8.3.20.1 Recipe: 5.0% w/v solution of silver nitrate in DI water.

8.3.20.2 Caution: Poison; will cause staining.

8.3.20.3 Storage: Store in the refrigerator in a dark environment.

8.3.20.4 Procedure: Dissolve sample in water. Add silver nitrate solution. A white, curdy precipitate will form in the presence of chloride ions which will be insoluble in nitric acid. The precipitate will be soluble in 6N ammonium hydroxide.

8.3.21 Stannous Chloride modification for $Co(SCN)_2$ - HCl acidified (¶ 8.4.9) differentiates between "caines".

8.3.21.1 Recipe: 5 g $SnCl_2$ and 10 mL conc. HCl diluted to 100 mL with H_2O

8.3.21.2 Procedure: The Stannous Chloride test is used as the second part of the Cobalt thiocyanate test (¶ 8.3.4). After performing the cobalt thiocyanate test, add a drop of stannous chloride reagent.

8.3.21.3 Results:

- Cocaine salts - blue remains
- Cocaine base - blue color forms (initially negative)
- Other compounds which turned blue initially - blue fades

8.3.22 Sulfuric Acid (H_2SO_4) (¶¶ 8.4.1, 8.4.2)

8.3.22.1 Recipe: concentrated sulfuric acid

8.3.22.2 Procedure: Add reagent to well and add sample.

8.3.22.3 Results:

- tetracycline - purple turning to yellow upon addition of water

- 2,3-MDMA, 2,3-MDA – rose
- 3,4-MDMA, 3,4-MDA – gray-brown

8.3.23 Tannic Acid (¶ 8.4.3) reacts with xanthines.

8.3.23.1 Recipe: 1% aqueous solution of tannic acid

8.3.23.2 Procedure: Add reagent to test tube then add powdered sample.

8.3.23.3 Results: caffeine and theophylline - positive test will produce a precipitate which develops from "streamers" immediately visible in the solution.

8.3.24 TBPEE Solution (¶ 8.4.8) differentiates between amines.

8.3.24.1 Recipe:

- 0.01g Tetrabromophenolphthalein ethyl ester (TBPEE) in 100 mL CCl_4
- 10.6 g sodium carbonate in 100 mL H_2O (2N solution)

8.3.24.2 Caution: Carbon tetrachloride is carcinogenic. Use appropriate safety precautions.

8.3.24.3 Procedure: Dissolve suspected amine in 2N Na_2CO_3 solution and add TBPEE solution. Note color change in the bottom TBPEE layer.

8.3.24.4 Results:

- primary amines - violet
- secondary amine - blue
- tertiary amine – red

8.3.25 Van Urk's (¶ 8.4.12) reacts with the indole moiety and some amines.

8.3.25.1 Recipe: 125 mg p-dimethylaminobenzaldehyde, 65 mL of concentrated H_2SO_4, and 2 drops of ferric chloride (USP T. S.) diluted to 100 mL with distilled water.

8.3.25.2 Procedure: Add reagent to well then add sample.

8.3.25.3 Results: LSD – blue/purple

8.3.26 Weber Test (¶ 8.4.6) reacts with psilocyn.

8.3.26.1 Recipe: 0.01 g of Fast Blue B or Fast Blue BB in 10 mL H_2O

8.3.26.2 Shelf life: One month from preparation.

8.3.26.3 Procedure: Add 2 to 3 drops of reagent to a sample of mushrooms. Observe slow color change. Add 1 to 2 drops of conc. HCl, observe color change.

8.3.26.4 Results: Psilocyn – Initially the solution turns red. The solution will turn from red to blue when the acid is added.

8.4 References

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9 THIN LAYER CHROMATOGRAPHY

9.1 Introduction

9.1.1 Thin layer chromatography (TLC) is a useful method for screening, separation and preliminary identification. Both the approximate concentration of the sample and the number of components contained in the sample can be ascertained by TLC. TLC can provide valuable information before proceeding on to instrumental tests. Clues as to the chemical structure of an analyte can be obtained by noting the distance traveled in different solvent systems and noting the reactions to a variety of chemical sprays.

9.1.2 The specificity of TLC is greatly increased by using multiple solvent systems of different polarities or pH. Because many drug compounds (or other organic compounds) have similar Rf values in any one solvent system, at least two solvent systems should be used, except for marijuana and hashish oil.

9.2 Materials

9.2.1 Solvent tank - Any covered glass container with a level bottom can be used. Rectangular tanks are most common. The developing solvent should be at a depth of approximately 0.5 cm to maintain constant contact with the stationary phase throughout the analysis. Filter paper or some other suitable absorbent paper should line the back inside wall of the tank at a height greater than the plate being used when it is required to maintain an atmosphere saturated with solvent vapor. This can provide for better migration and more consistent results. Care should be taken to maintain this atmosphere. The absorbent paper is not required for marijuana analysis.

9.2.2 Thin layer plates - silica gel (250µm) coated glass plates with a fluorescent indicator, or equivalent (Most drug compounds quench fluorescence when visualized under short wave UV light)

9.2.3 Capillary tubes or Micropipettes

9.2.4 Long wave/short wave UV light source

9.2.5 Solvent baths

9.2.6 Visualization reagents

9.3 Methods

9.3.1 The sample to be tested is dissolved in $CHCl_3$, MeOH or other suitable solvent. The solvent used must be recorded in the case notes.

9.3.2 The solution is drawn up into a capillary tube and 1-10 µl (depending on concentration) is spotted on a dry plate approximately 0.5 - 1 cm from the bottom, making sure that the spot is above the solvent level in the developing tank. The spot size should be kept to a minimum as its diameter will increase while the compound migrates up the plate during development. Heavy concentrations should be avoided as this causes streaking and tailing.

9.3.3 A standard is spotted beside the sample(s) for comparison. Care should be taken that the standard and sample(s) are approximately the same concentration. Unequal concentrations may result in unequal rates of advance. This can easily be checked by visualizing the plate under UV light before development.

9.3.4 A blank of the solvent used to dissolve the sample is also spotted on the plate. The results of the blank must be recorded in the case notes. This may be done by using a check mark (√) or "ok" or "-" to record that the results of the blank were acceptable (e.g., Blk √).

9.3.4.1 If spots are visualized in the blank region, the blank should be run again under the same conditions.

9.3.4.1.1 If the results of the second blank are acceptable, the entire plate should be re-spotted and re-run.

9.3.4.1.2 If the results of the second blank are unacceptable, the analyst should take steps to resolve the issue (e.g., replacing the solvents in the bottle, checking the baths) prior to re-sampling and any further analysis.

9.3.5 The plate is placed in the tank and allowed to develop until the solvent reaches the top. The plate is then removed, dried, inspected under UV light, and/or sprayed with the appropriate visualizing reagent. Do not allow the plate to stand in the solvent after development is complete as this will cause a gradual diffusion of the compound.

9.3.6 Specific solvent systems and developing sprays utilized in casework will be denoted in the analytical case notes. Positive TLC results may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) along with the standard used in the comparison. A result is considered positive when the distance traveled and the reaction with the visualization methods compare favorably with a standard. Negative reactions may be recorded in a similar fashion; standards used for which negative results are observed need not be documented.

9.4 TLC Baths

- TLC1: $CHCl_3/CH_3OH$ (9:1)(v/v) – general drug screening (¶ 9.8.1)
- TLC2: Ammonia washed $CHCl_3/CH_3OH$ - (18:1) (v/v) – general drug screening (¶ 9.8.2)
- TLC3: T-1 Methanol/NH_4OH (100:1.5) (v/v) – general drug screening (¶ 9.8.1)
- TLC4: 8% Diethylamine in Toluene (v/v) – marijuana / general drug screening (¶ 9.8.3)
- TLC5: 4% Diethylamine in Toluene (v/v) – marijuana / general drug screening (¶ 9.8.3)
- TLC6: Chloroform/Ethyl Acetate (80:20) (v/v) – steroids (¶ 9.8.5)
- TLC7: Isopropyl Ether – barbiturates (¶ 9.8.8)
- TLC8: Acetone /$CHCl_3$ (2:1)(v/v) – LSD/LAMPA (¶ 9.8.2)
- TLC9: Cyclohexane/Toluene/Diethylamine (75:15:10) (v/v) – MDMA/methamphetamine (¶ 9.8.1)
- TLC10: Ethyl Acetate – GHB, GBL, 1,4-butanediol
- TLC11: Deionized water - vitamins
- TLC12: Acetone – amphetamines and other basic drugs (¶ 9.8.4)
- TLC13: Ethyl acetate/acetone/ammonium hydroxide (25:5:1) (v/v) – ephedrine/pseudoephedrine
- TLC14: Ethyl acetate/hexane (1:1) (v/v) – salvinorin A (¶ 9.8.11)

9.5 Visualization Reagents

9.5.1 All visualization sprays must be used in a fume hood.

9.5.2 Ceric Sulfate (¶ 9.8.10)

9.5.2.1 Used as an overspray to intensify the reaction with iodoplatinate, especially for caffeine

9.5.2.2 Recipe: 5 g $Ce(SO_4)_2$ in 500 mL H_2O and 14 mL H_2SO_4.

9.5.3 Diphenylcarbazone (¶ 9.8.6)

9.5.3.1 Used as an overspray with mercuric sulfate for barbiturates. Can also be freshly mixed 50/50 with the mercuric sulfate reagent (¶ 9.5.12).

9.5.3.2 Recipe: 19 mg diphenylcarbazone in 200 mL (50% acetone/water).

9.5.4 Dragendorff (¶ 9.8.1)

9.5.4.1 General spray which visualizes alkaloids and other nitrogen containing compounds, including methamphetamine and diazepam.

9.5.4.2 Recipe: 1.3 g of bismuth subnitrate in 60 mL water with 15 mL glacial acetic acid. Add this to 12 g potassium iodide in 30 mL H_2O. Dilute with 100 mL of H_2O and 25 mL glacial acetic acid.

9.5.5 Ehrlich's or p-Dimethylaminobenzaldehyde (p-DMAB) (¶ 9.8.7)

9.5.5.1 Visualizes LSD and psilocybin, reacts with indole nucleus of alkaloids. Plate may be heated after spraying to increase intensity of color.

9.5.5.2 Recipe: 2 g of p-DMAB in 50 mL 95% ethanol and 50 mL 37% HCl.

9.5.6 Fast Blue B (¶ 9.8.1) or Fast Blue BB (¶ 9.8.9)

9.5.6.1 Visualizes the three major cannabinoids in marijuana. They migrate and develop in the following order:

- Top spot - Cannabidiol – orange
- Middle spot - Tetrahydrocannabinol (Δ^9-THC) - red
- Lower spot - Cannabinol – purple

9.5.6.2 Visualizes psilocyn - red which then turns blue when acidified with HCl.

9.5.6.3 Recipe: Approximately 0.05% solution of Fast Blue B salt OR Fast Blue BB salt in water

9.5.6.4 Shelf life: One month from preparation.

9.5.7 Fluorescamine (Fluram^R)

9.5.7.1 Visualizes amino acids, primary amines and amino sugars.

9.5.7.2 Recipe: 20 mg Fluram^R in 100 mL acetone.

9.5.7.3 Procedure: Spray plate with reagent, then check under long wave UV light (amphetamine fluoresces green-yellow). Heating the plate may intensify the visualization.

9.5.8 Furfuraldehyde and HCl (¶ 9.8.1)

9.5.8.1 Visualizes meprobamate and other carbamates.

9.5.8.2 Recipe: 10% solution of furfuraldehyde in ethanol. Overspray with concentrated HCl.

9.5.8.3 Procedure: Spray plate and heat, if necessary. Spots are black on a white background.

9.5.9 6N HCl

9.5.9.1 Used to acidify plates

9.5.10 Iodine Vapors (¶ 9.8.1)

9.5.10.1 Visualizes general unknowns and compounds which are not UV active. Suitable for GHB, 1,4-butanediol and GBL analysis. This is a good method of visualization if further testing is to be done on the sample on the plate, as it is reversible.

9.5.10.2 Procedure: Place iodine crystals in an enclosed chamber. Let TLC plate develop in the chamber. Many organic compounds will produce a brown spot.

9.5.10.3 Results:

- GHB – white spot on yellow background
- GBL, 1,4-butanediol – brown spot on yellow background.

9.5.11 Iodoplatinate (¶ 9.8.1)

9.5.11.1 Visualizes nitrogen-containing compounds, may be acidified with HCl to intensify some reactions.

9.5.11.2 Recipes:

- 5 mL of 10% platinic chloride aqueous solution and 10 g of potassium iodide in 500 mL of H_2O.
- 1 g of platinic chloride and 10 g of potassium iodide in 500 mL H_2O.

9.5.11.3 If acidified iodoplatinate is preferred, either overspray the TLC plate with 6 N HCl or prepare stock solution with approximately 5% HCl.

9.5.11.4 Results may be intensified with an overspray of ceric sulfate reagent.

9.5.12 Mercuric Sulfate (¶ 9.8.8)

9.5.12.1 Visualizes barbiturates, which appear as white spots on off-white background. The plate may need to be sprayed heavily.

9.5.12.2 Recipe: Suspend 5 g HgO in 100 mL of H_2O. Add 20 mL concentrated H_2SO_4. Cool, dilute with 250 mL H_2O.

9.5.12.3 Mercuric Sulfate can also be freshly mixed 50/50 with the diphenylcarbazone reagent (¶ 9.5.3).

9.5.13 Ninhydrin (¶ 9.8.1)

9.5.13.1 Visualizes amino acid, primary and some secondary amines and amine sugars. (¶ 9.8.1)

9.5.13.2 Recipe: Add 0.5 gram of ninhydrin to 10 mL concentrated HCl. Dilute to 100 mL with acetone.

9.5.13.3 Procedure: Spray with ninhydrin solution and heat the plate (e.g., hotplate, approximately 100 C oven) for 2 minutes. After spraying, the plate may be irradiated under long wave UV light for 2 minutes prior to heating.

9.5.13.4 Results: yields pink-violet or orange-brown spots.

9.5.13.5 Alternatively, the commercially available Chem Print Ninhydrin may be used.

9.5.14 Potassium Permanganate (¶ 9.8.1)

9.5.14.1 Visualizes unsaturated hydrocarbons. $KMnO_4$ is an alternative to Mercuric Sulfate for barbiturates which contain a double bond. $KMnO_4$ may be used as an underspray or overspray with Iodoplatinate.

9.5.14.2 Recipe: Dissolve 1 g $KMnO_4$ in 100 mL H_2O.

9.5.14.3 Results: yields a yellow spot on a purple background.

9.5.15 Sulfuric Acid/Ethanol Reagent for Steroids (¶ 9.8.5)

9.5.15.1 Recipe: Add gradually 10 mL of conc. sulfuric acid to 90 mL of ethanol.

9.5.15.2 Procedure: Spray plate and heat gently on a hot plate to develop.

9.5.15.3 Results:

- Testosterone - green
- Testosterone esters – purple
- Oxymethalone – red
- Nandrolone decanoate – purple

9.5.16 Vanillin Reagent for Salvinorin A (¶ 9.8.11)

9.5.16.1 Recipe: 1 g of vanillin with 50 mL anhydrous ethanol and 0.3 mL concentrated H_2SO_4

9.5.16.2 Procedure: After developing plate, dry thoroughly. For plate developed in the basic TLC2 bath, spray plate with 6N HCl prior to Vanillin. Spray plate generously with reagent and heat with heat gun for approximately 2 minutes or place in oven at 110 °C for several minutes.

9.5.16.3 Results: Salvinorin A - Pink/Purple spot. Marijuana also gives a pink/purple spot, but at a different R_f when using TLC14.

9.5.16.4 Store in refrigerator when not in use.

9.6 Preparative Thin Layer Chromatography

9.6.1 Introduction

9.6.1.1 Frequently, samples contain other organic compounds which interfere with the drug analysis (e.g., heroin and quinine). Preparative TLC can be used to clean up a sample for other methods of testing such as IR or MS.

9.6.1.2 If cleaning up cocaine for a base determination, be careful to use a neutral bath to develop the plate so that the original salt form will not be altered.

9.6.2 Materials

9.6.2.1 Thin layer plates

9.6.2.1.1 A section of 250µm thin layer plate can be used if only a small quantity of pure compound is needed.

9.6.2.1.2 For larger quantities, use a 1000µm plate.

9.6.3 Procedure

9.6.3.1 The sample is dissolved in an appropriate solvent and streaked along the bottom of the plate using a capillary tube, long tipped Pasteur pipette or a commercial streaking device (if available).

9.6.3.2 A standard may be spotted separately at either the beginning or end of the plate in order to identify the desired compound after development.

9.6.3.3 Develop the plate as in regular TLC.

9.6.3.4 After drying the plate, the desired area is located and marked under UV light. (For compounds not UV visible, iodine vapors can be used.)

9.6.3.5 Scrape off the desired area, wash thoroughly with solvent in a small beaker and filter to remove the silica gel. Smaller quantities can be filtered using a disposable Pasteur pipette with a pre-washed glass wool plug.

9.6.3.5.1 After development, most compounds adhere strongly to the deactivated silica gel and therefore must be washed with a fairly polar solvent. Methanol is recommended. For some compounds, an extraction from an aqueous acidic or basic solution may be necessary.

9.6.3.6 If using two-dimensional TLC, first develop the plate as usual, and then develop the plate in a polar solvent system at a 90 degree angle in order to concentrate the sample into a tighter spot. The standard would need to be removed by breaking off the portion of the plate containing the standard prior to this step. The compound is then removed from the silica as described above in Section 9.6.3.5.

9.7 Comparative Semi-Quantitative Thin Layer Chromatography

9.7.1 Thin layer chromatography can be used to determine relative concentration between a sample and a standard. This is useful when it is necessary to determine whether a pharmaceutical preparation has been diluted or substituted. In cases where an exact assay is needed, a suitable quantitation should be performed.

9.7.2 Procedure:

9.7.2.1 Obtain or prepare a standard at the concentration expected for the sample.

9.7.2.2 Apply equal amounts of the standard solution and the sample solution to the TLC plate.

9.7.2.3 Develop and visualize the plate as described above for regular TLC.

9.7.2.4 Visually compare the size and color of the spots to determine if the substance has been substituted or diluted.

9.7.2.5 Approximate concentrations can be estimated by bracketing the observed sample concentration within appropriate standard dilutions. Visually compare the sample response to that of the closest standard dilution. This approximation should be recorded in the case notes but not indicated on the report.

9.7.3 Reporting: Any controlled substance present will be initially identified in the usual manner. The concentration or substitution of the sample will normally be addressed in the report with one of the two following statements for suspected tampering cases.

9.7.3.1 Meets label specifications (with regard to concentration and/or contents).

9.7.3.2 Does not meet label specifications (with regard to concentration and/or contents).

9.7.3.3 Any pharmaceutical substitutions or adulterants, properly identified, should be reported as usual. Their concentrations will not normally be required. (e.g., Diazepam substituted into a device labeled to contain morphine would be identified and reported with no concentration value required).

9.8 References

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10 GAS CHROMATOGRAPHY

10.1 Introduction

10.1.1 Gas Chromatography (GC) is a useful method for screening, separation and preliminary identification. GC provides both qualitative and quantitative information about the components of samples. Specificity is dependent on a variety of factors including stationary phase and type of detector. GC can be used to determine such things as isomers along with sufficient structurally definitive information from other techniques.

10.1.2 GC retention times of the analyte are compared to that of a known standard. The specificity of GC is increased by using two columns with stationary phases of different polarities.

10.1.3 Specific column designations, conditions and detectors utilized in casework will be denoted in the analytical case file. Positive GC results may be recorded in the analytical notes by the use of a plus (+), a plus circled (⊕) or an abbreviation (e.g., pos) along with the standard used in the comparison.

10.2 Materials

10.2.1 Capillary Columns:

10.2.1.1 All routine methods employ gas chromatography using flexible fused silica capillary columns of 0.20 to 0.320 mm i.d.

10.2.1.2 The stationary phase is chosen to effect needed resolution. Methylsilicone (e.g., HP-1) and 5% phenylmethyl silicone (e.g., HP-5 and HP-5MS) are utilized in routine casework. The film thickness should be approximately 0.25 microns. The normal general purpose column has a 0.25 μm film thickness and 0.25 mm internal diameter. (35% phenyl)-methylpolysiloxane (e.g., HP-35 and HP-35MS) columns may be used when increased polarity is beneficial for separating compounds with similar structures, such as synthetic cannabinoids.

10.2.1.3 All routine methods utilize columns containing a bonded, cross-linked stationary phase.

10.2.1.4 If a stationary phase is required that is outside of these recommended parameters for a specialized analysis or if more resolving power is required, an alternative column may be used or an additional, different diameter and/or phase column can be temporarily attached at the end of the existing column by using an appropriate connector.

10.2.1.4.1 These changes should be made under the guidance of the instrument operator and must be approved by the Chemistry Program Manager.

10.2.1.4.2 In the case of thick films or non-bonded stationary phases, the extra column bleed generated may require more frequent maintenance of the detector.

10.2.2 Additional Instrument Parameters

10.2.2.1 The carrier gas is normally a high purity helium at a flow rate of 0.5 to 3 mL/min.

10.2.2.2 Nitrogen makeup gas is recommended in order to support gas flow at the FID to provide optimal detector sensitivity.

10.2.2.3 Split/splitless liners designed specifically for use with the particular instrument should be used.

10.2.2.3.1 Injection port liners may be reused after appropriate cleaning and deactivation.

10.2.2.4 For “open tubular" liners, a small amount of silanized glass wool shall be inserted in the center of the liner.

10.2.2.4.1 If the liner is to be packed with GC column packing material, the packing material should be sandwiched between layers of silanized glass wool or equivalent. Packed liners may require "on column" silyl treatment when first installed.

10.2.2.4.2 Liner packing material: The solid support should be either Chromasorb W-HP or Gas Chrom Q. Mesh size should be 80/100 or 100/120 mesh. Stationary phases such as OV-1, OV-17, OV-7, and SE 30 series or their equivalent at 2-5% loading may be utilized.

10.2.2.5 The use of a "two hole" capillary ferrule allows two capillary columns of slightly different polarities to be connected into the same injection port. The sample is analyzed on two columns with a single injection of typically less than 5 µL.

10.2.2.6 Detectors most appropriate for normal drug analysis include both flame ionization detectors and mass spectrometers. Other specific detectors such as NPD and ECD may be used in circumstances requiring them in consultation with the Chemistry Program Manager. Retention time comparison may be accomplished with any detector. Quantitative analyses should use the flame ionization detector.

10.3 Methods

10.3.1 Analysis conditions are generally set to allow for sample elution time to be greater than 3 - 5 times that of the solvent front. This allows the sample to interact sufficiently with the stationary phase.

10.3.2 The maximum allowable temperature program ramp rate for reproducible retention times is 30 degrees centigrade per minute for Agilent GC's Model 6890 and higher.

10.3.3 In most instances injection is made in the split mode at a split ratio of 5 - 100:1. Splitless injections may be used when required to increase the amount of analyte delivered to the column and the detector.

10.3.4 Normal injection volume and sample size should be sufficient to provide 8 - 160 nanograms of analyte "on column" for the normal setup. This correlates to a 1 µL injection of an approximate range of solution concentrations of 0.5 – 10 mg/mL, based on a typical 60:1 split ratio.

10.3.5 Samples should be dissolved in n-hexane, CH_2Cl_2, $CHCl_3$, ammonia saturated $CHCl_3$ or MeOH for GC analysis. Depending on the nature of the samples, some samples must be cleaned up by extraction, but most may be directly dissolved in the solvent.

10.3.6 Sample concentrations should be approximately the same concentration as the GC standard and should be within the linear dynamic range of the chromatographic system and detector.

10.3.7 For comparison purposes, a standard must be run using the same method conditions as the samples. Standards used in the comparison must be run on the same day as the sample. "Same day" is defined as an approximate 24 hour period.

10.3.8 At a minimum, a blank consisting of the solvent(s) used to dissolve the samples, must be run on both the GC and GC/MS systems, when any of the following conditions are met:

- Before each analyst's series of sample runs whether manual or autosampler methods are utilized.
- No more than 10 samples can be run before another blank or standard/blank combination is required. A sample's position relative to the blank shall be documented in the case file. This may be accomplished by several methods, including consecutive data file numbering when using "windows macros".

- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded (except those that merely change the injection volume or split ratio) or run between blank and sample.

- It is strongly suggested that a solvent blank be injected and properly documented immediately prior to a sample known to be extremely weak.

- Additional blanks may be run at the examiner's discretion.

- The injection order when running samples with standards should be either "standard, blank, sample(s)" or "blank, sample(s), standard."

10.3.8.1 The solvent blank must be of at least as large an injection volume of the same solvent as the sample to be injected. The upper limit injection volume is normally 4 µL.

10.3.8.2 The solvent blank must be run at the same or lower split ratio as the sample. The solvent blank shall be run directly before samples which are run at a reduced split ratio (e.g., 20:1 when typical methods use 60:1).

10.3.8.3 Any significant peaks in blank chromatograms must be properly investigated and documented in the referenced case file.

10.3.8.3.1 If a controlled substance or related compound is present in any concentration, the blanks and associated samples should be re-run.

10.3.8.3.2 If an interfering substance is present, the blanks and associated samples should be re-run.

10.3.8.3.3 Blanks and associated samples should be replaced and re-sampled, respectively, prior to further analysis if the same extraneous peaks are still present.

10.3.9 In all instances, the GC standards file may be referred to for chromatographic conditions. Broad screening methods can be surmised from these files.

10.3.10 Sequencing via autosampler should be utilized whenever practical.

10.3.11 Sequences and/or samples (including standards and blanks) shall be recorded in the instrument logbook.

10.3.11.1 Data files should not be overwritten.

10.3.11.2 Sequence files should not be overwritten unless additional data files have been added during the sequence run.

10.3.11.3 Sequences and sequence log files shall be archived along with data files as per ¶ 35.6.4.2.

10.3.12 Integrated retention times for analytes must agree with the standard within 2 seconds (± 2 sec.) or 0.033 minutes for this to be considered a positive result.

10.3.13 Derivatization

10.3.13.1 Some compounds, such as amphetamines or barbiturates, do not chromatograph well. Derivatives may need to be made to help effect good chromatographic peak shape.

10.3.13.2 Procedures:

10.3.13.2.1 Acetyl derivatives – appropriate for primary and secondary amines

- The acetyl derivative of phenethylamines is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. Acetyl derivatives generally have a longer retention time than the underivatized compound and may require a higher temperature than the underivatized compound.

- These derivatives can also be formed prior to injection by heating the sample and derivatizing reagent (~ 70° C) in a closed vial.

10.3.13.2.2 Alkyl derivatives – appropriate for barbiturates

The methyl derivative of barbiturates is made by the same procedure as listed above, only using trimethylanilinium hydroxide (TMAH) instead of acetic anhydride. Methyl derivatives often have a shorter retention time and may require a lower temperature than the underivatized compound.

10.3.13.2.3 Silyl derivatives

10.3.13.2.3.1 Silyl derivatives are often very helpful in the analysis of compounds that exhibit chromatographic difficulties due to polar functional groups such as alcohols, amines, acids, and phenols (e.g., GHB, morphine). Silyl derivatives exhibit an M-15 and M-57 peak and sometimes do not exhibit a molecular ion peak in electron impact (EI) mass spectrometry.

10.3.13.2.3.2 There are several good silylation reagents available from Regis Chemical Co., Pierce Chemical Co. and others which are designed for various applications. Catalogues from these companies are quite useful in determining the most useful derivatizing agent and their application procedure. BSTFA and BSTFA with 1% TMCS work well as silylating reagents for drug compounds.

10.3.13.2.3.3 A suitable aprotic solvent (e.g., pyridine, chloroform, toluene) may be used to dissolve the analyte and the manufacturers' directions must be followed carefully to achieve the desired result.

10.3.14 Split/Splitless Liner Cleaning and Preparation Methods

10.3.14.1 Liner Cleaning with aluminum oxide

- Remove any packing material.
- Dip cotton swab in aluminum oxide slurry and twist swab into liners to remove residue.
- Sonicate liners in water with laboratory glassware soap, rinse several times with water, acetone and then methanol.
- Dry thoroughly.
- Immerse liners in silylating reagent (10% dichlorodimethylsilane in toluene) and let stand at least 2 hours, preferably overnight.
- Rinse three times with toluene and dry thoroughly.
- Wear nylon gloves when handling clean liners and glass wool.
- Insert packing, such as silanized glass wool, prior to installation in the instrument.
- After installation, put injection port and oven temperatures to 290 °C. Allow to sit for at least five minutes at this temperature before running QA mix.

10.3.14.2 Liner Cleaning with HCl

- Remove any packing or glass wool.
- Soak liners in 1N HCl for at least eight hours (overnight is fine).
- Rinse with distilled water and methanol.
- After thoroughly drying, soak liners in silylating reagent (10% dichlorodimethylsilane in toluene) and let stand at least 2 hours, preferably overnight.
- Rinse three times with toluene and dry thoroughly.
- Wear nylon gloves when handling clean liners and glass wool.
- Insert packing, such as silanized glass wool, prior to installation in the instrument.
- After installation, put injection port and oven temperatures to 290 °C. Allow to sit for at least five minutes at this temperature before running QA mix.

10.4 Quantitation

10.4.1 Gas Chromatography utilizing a flame ionization detector is an excellent method for quantitative analysis. The preferred method is the internal standard method.

10.4.2 The Department does not require routine quantitation of drugs. When it is specifically requested and required to assay a sample, this is the general procedure for a suitable GC quantitation method. Specific examples are found in the sections specific to a particular compound.

10.4.3 Validation

10.4.3.1 Select an appropriate internal standard which will not coelute with components of the sample. Ideally, this internal standard should elute prior to the analyte of interest. In instances where this is not practical, an internal standard with a similar elution time, though later, may be chosen.

10.4.3.2 Run standards at 4 or 5 different concentrations over the range of interest. The target mid-range is 1.0 mg/mL. All instrument conditions must remain constant over the range. Inject each standard at least three times. A methylsilicone (e.g., HP-1) column with a 0.25 μm film thickness, 0.25 mm internal diameter and approximate 15 m length will be utilized for quantitative methods.

10.4.3.3 The low standard will define the method's lower limit of quantitation. The high standard will define the upper limit of quantitation.

10.4.3.4 Average the response ratios for each standard and calculate the best fit of average response ratio (y-axis) vs. concentration (x-axis) using linear regression using the *Linearity Worksheet – 4 points* or the *Linearity Worksheet – 5 points*. The plot of the fit must appear linear. The regression coefficient (R^2) must be greater than or equal to 0.99.

10.4.3.5 Calculations

10.4.3.5.1 Back calculate the apparent concentration of each injection using the linear regression equation from the plot.

10.4.3.5.2 Calculate the mean and % standard deviation of the apparent concentrations for each standard.

10.4.3.5.3 Calculate the % difference of each mean from the known concentration of the corresponding standard; each value (the accuracy) must be less than or equal to 10%.

$$\% \text{ Difference (Accuracy)} = \frac{[\text{Calculated}] - [\text{Theoretical}]}{[\text{Theoretical}]} * 100$$

10.4.3.5.4 Each % standard deviation (the precision) must be less than or equal to 3%.

10.4.3.6 The data and calculations for each validation will be kept in the lab of origin and a copy will be sent to the Chemistry Program Manager for approval.

10.4.4 General Quantitation Procedure

10.4.4.1 Weights will be measured using an analytical balance with a readability of +/- 0.0001 gram. Quantities used in the preparation of standards and sample for quantitative purposes shall be at or above 10 milligrams (0.0100 gram).

10.4.4.2 Solutions will be made up in Class A volumetric flasks. When exact volumes are required for extractions, Class A volumetric pipettes shall be used. Graduated pipettes are not acceptable for quantitative purposes.

10.4.4.3 Make up an internal standard solution of known concentration between 1-2 mg/mL, which will be used in making all standard and sample solutions. Refrigerated solutions should be allowed to return to ambient temperature prior to use.

10.4.4.4 Make up two standard solutions of different concentrations within the acceptable linear range (e.g., 1 mg/mL and 3 mg/mL) in the internal standard solution as defined in the method. Do not use serial dilutions.

10.4.4.4.1 One of these standards will act as the standard for the one point calibration calculation and the other will serve as a check standard (control).

10.4.4.4.2 The calibration standard shall be chosen as the one closest in concentration to the unknown samples.

10.4.4.5 All quantitations will be done in duplicate. Prepare two separate sample solutions as per the method. Refer to Quantitative Sampling (¶ 5.8) for further information.

10.4.4.6 Run the two standards, a blank of the internal standard solution, and the duplicate samples using the appropriate GC method. The standards and samples will be injected three times and the ratios will be averaged to calculate the concentration. One injection of the blank is sufficient. The injection volume should be 1-2 µl.

10.4.4.7 Using the equation listed below, calculate the % purity of both the check standard and the samples. The *Quantitation Worksheet* can be used for this purpose.

10.4.4.7.1 The concentration of the check standard must be within 10% of the theoretical value.

10.4.4.7.2 The precision of the check standard must be within 3%.

10.4.4.7.3 The results from the duplicate samples must be within 10% of each other.

10.4.4.7.4 If the above criteria are met, the results may be reported.

10.4.4.7.5 If any of the above criteria are not met, take appropriate steps (e.g., perform appropriate corrective instrument maintenance, remake both of the standard solutions and repeat, reevaluate the linearity of the instrument) to resolve the problem.

10.4.4.8 Standard Salt Form Calculation

10.4.4.8.1 Unless the salt form of the drug is to be reported, the concentration of the analyte in its base form will be calculated and reported. If the calibration standard is not in free base form, it will need to be corrected.

10.4.4.8.2 Example: Heroin hydrochloride monohydrate is used to quantitate a sample containing heroin. The sample will be reported as "Heroin" without a specified salt form. The calibration standard is made up using 22.4 mg of heroin hydrochloride in 10.0 mL of internal standard solution. The corrected concentration will be:

[Heroin base] = (22.4 mg ÷ 10.0 mL) * (369.4 g/mol ÷ 423.9 g/mol)

[Heroin base] = 1.95 mg/mL

10.4.4.9 Purity Calculation

$$\% \text{ Drug} = \frac{[STD] \times R_2 \times V}{R_1 \times W} \times 100$$

[STD] = concentration of calibration standard in mg/mL

R_2 = peak area (height) of sample / peak area (height) of internal standard

R_1 = peak area (height) of standard / peak area (height) of internal standard

V = volume of internal standard solution used in mL

W = sample weight in mg

10.4.4.10 Reporting

10.4.4.10.1 Take the lower of the duplicate analyses and truncate to the whole integer. Once the UOM policy has been implemented, subtract the estimated uncertainty value prior to truncating.

10.4.4.10.2 If the purity is calculated as the salt, the salt form of the drug should be reported.

10.4.4.10.3 The "show form" option will be utilized in LIMS.

10.4.4.10.4 Examples:

- 24.55 grams of solid material, found to contain Heroin (Schedule I), 26% pure.
- 12.25 grams of powder, found to contain Cocaine Hydrochloride (Schedule II), 45% pure.

11 ULTRAVIOLET SPECTROSCOPY

11.1 Introduction

11.1.1 Ultraviolet spectroscopy (UV) is a good preliminary screening method for identifying an organic compound with aromatic rings or conjugated systems. However, this method has limited specificity because structurally related compounds give similar spectra.

11.1.2 Light energy absorbed in the ultraviolet region causes the electrons to undergo transitions from ground states to higher-energy states (electronic transitions). These transitions and the corresponding wavelengths of absorbed energy are characteristic of a group of atoms called a chromophore. Addition of substituents with electron withdrawing or donating properties to the conjugated system of the chromophore causes changes in the resulting spectrum.

11.1.3 In UV spectroscopy, the sample is placed between an energy source (Deuterium lamp) and a spectrometer. The source provides electromagnetic radiation in the ultraviolet region (180 - 400 nm). Many UV spectrophotometers are double beam instruments, in which the spectrometer measures the absorbed energy relative to a reference. The reference beam serves to subtract solvent absorptions from the resulting spectrum. Other instruments which contain diode array detectors (e.g., scanning LC detectors) are also allowable.

11.1.4 The choice of solvent is important. The solvent should not absorb ultraviolet radiation in the same region as the sample. Solvents without conjugation such as water (range of pH), 95% ethanol, and n-hexane are most commonly used.

11.1.4.1 These solvents vary as to the shortest wavelength at which they remain transparent to UV radiation.

11.1.4.2 The effects of a solvent on the fine structure of an absorption band should be considered. For example, in a polar solvent the hydrogen bonding forms a solute-solvent complex and the fine structure may disappear.

11.1.4.3 Sometimes the spectrum in an acidic medium is different from the spectrum in a basic medium. The change in absorption maxima due to a change in pH can be indicative of certain compounds. Examples of compounds which exhibit a distinctive acid-base shift are acetaminophen, morphine, and most barbiturates.

11.1.5 UV spectroscopy can be very useful as a quantitation technique as it is based on the Beer-Lambert Law.

11.2 Sample Preparation

11.2.1 For drug screening, dissolve the sample in both acidic and basic media. For most drug analyses, suitable solvents are 0.2N H_2SO_4, 0.1N HCl and 0.1N NaOH.

11.2.2 Samples should be relatively pure for UV quantitation. Samples containing more than one absorbing compound may have additive absorbance values. Extraction is the most common method for purification. Samples containing other substances that do not absorb in the UV region of interest need not be removed prior to quantitation.

12 ANALYSIS OF PHARMACEUTICAL INJECTABLE DOSAGE FORMS

12.1 Introduction

12.1.1 The analysis of cases involving pharmaceutical injectable dosage forms may require more than just the identification of the contents.

12.1.2 Tampering can involve the dilution, contamination, or removal of the contents of a Tubex®, Carpuject®, injection vial, pharmacy intravenous (IV) preparation, IV stock solution, or IV supplies and apparatus.

12.2 Procedure

12.2.1 Carefully visually inspect the item. Note stopper condition (for small punctures), plunger location and condition, fill volume, appearance, color and consistency of the contents.

12.2.2 If possible, a standard of the same brand and lot number should be requested from the submitting agency or a licensed pharmacy for comparison of the visual characteristics, chemical contents and concentration.

12.2.3 Any controlled substance present will be identified in the usual manner.

12.2.4 Analyses for concentration are normally run with semi-quantitative TLC, comparative UV quantitation, or standard GC quantitation methods.

12.3 Reporting

12.3.1 The concentration of the sample will normally be addressed in the report with one of the two following statements for suspected tampering cases. Additional clarifying wording may be used at the discretion of the Section Supervisor, such as "Does not meet label specifications with regard to concentration" or "Does not meet label specifications with regard to concentration and contents."

12.3.1.1 Meets label specifications.

12.3.1.2 Does not meet label specifications.

12.3.1.3 Any pharmaceutical substitutions or adulterants, properly identified, should be reported as usual. Their concentrations will not normally be required. (e.g., Diazepam substituted into a device labeled to contain morphine would be identified and reported with no concentration value required).

13 INFRARED SPECTROSCOPY

13.1 Introduction

13.1.1 Infrared spectroscopy (IR) is a specific method of identification in most instances and is therefore a desirable analytical tool for the forensic drug chemist. IR may be used to obtain semiquantitative data on known mixtures to express relative percentages, but is not normally used for quantitation.

13.1.2 This method of spectral analysis is based on the molecular vibrational energies of an organic compound. Infrared light containing wavelengths from 4000 cm^{-1} to 400 cm^{-1} is generated and passed through the sample. When the frequency of light matches a frequency of vibration within the molecule, absorption occurs. The absorptions are translated electronically and recorded on a data system. The resulting spectrum will have characteristic bands corresponding to each different vibration among atoms in the molecule.

13.1.3 The IR spectrum of an unknown compound can be compared to the IR spectrum of a known or suitable reference spectrum for confirmation.

13.1.4 The Fourier Transform Infrared Spectrophotometer (FTIR) collects the composite spectrum in the time domain and mathematically transforms it to the frequency domain.

13.1.5 Non-chemical separations (spectral subtraction) may be performed to determine components of a mixture. The components would need to be separated and structural confirmation of the pure compounds done by this or other structural identification techniques, if needed.

13.1.6 Spectra may be collected using an Attenuated Total Reflectance (ATR) accessory and compared to standards also collected utilizing the ATR. These standards may be stored in a user generated library. For unknown compounds, an ATR correction may be utilized in order to search a library of transmission spectra. The uncorrected unknown spectrum would then be compared to that of a known uncorrected standard spectrum.

13.1.7 If unique sample preparation or data reduction techniques are required, consult the Primary Operator for the FTIR.

13.2 Sample Preparation

13.2.1 Samples should be relatively pure and can be cleaned up by extraction, preparative TLC, recrystallization, or precipitation and filtration, depending upon the quantity and type of contaminants present.

13.2.2 Pure liquid organics can be run neat between two salt (NaCl) plates or using the ATR accessory.

13.2.3 Pure solids can be dissolved in a suitable organic solvent and run in solution cells, mixed with KBr and pressed into a pellet, mixed with a saturated long chain hydrocarbon oil (mulled) or run using the ATR accessory.

13.2.4 Solution Technique

13.2.4.1 A small amount of the sample is dissolved in a non-polar solvent such as CCl_4 or CS_2. Polar solvents such as MeOH or EtOH should be avoided. Other slightly polar solvents, such as $CHCl_3$, can also be used but will have some interfering absorption bands due to C-H.

13.2.4.2 Oils or dissolved solids may be deposited or "cast" on a salt plate (e.g., standard NaCl window) and placed in the sample beam. (Care must be taken to drive off all residual solvent).

13.2.4.3 The solvent absorption bands may be subtracted from the spectrum. Either a pair of salt plates with the solution solvent or a solution cell (of the same pathlength) containing only solvent can be scanned into the background spectrum.

13.2.5 Gas Techniques

13.2.5.1 Standard 10 cm gas cells or other similar hardware (e.g., multiple internal reflectance units) can be used.

13.2.6 Mull Technique

13.2.6.1 The sample is finely ground and suspended in mineral oil (Nujol). A thin film of the suspension is placed between two salt plates.

13.2.7 Pellet Technique

13.2.7.1 Infrared grade KBr should be kept dry by storing it in a suitable location such as a dessicator.

13.2.7.2 Infrared grade KBr and the sample each must be finely ground. The KBr and sample are mixed by grinding with a mortar and pestle in an approximate ratio of 100 parts KBr to 1 part sample.

13.2.7.3 The mixture is placed in a pellet press to prepare the pellet. A hand press with a 7 mm die or the Hydraulic 13 mm die set may be used. The 7 mm hand press KBr pellet is the preferred preparation technique.

13.2.8 ATR Accessory

13.2.8.1 Clean the diamond crystal and sapphire anvil surface before and after analysis with acetone or methanol soaked wipes. Methanol takes a slightly longer time to evaporate.

13.2.8.2 A background is collected prior to each sample.

13.2.8.2.1 An acceptable background shall be noted on the data.

13.2.8.2.2 If unexpected peaks are present in the background, the ATR crystal and anvil shall be cleaned and the background repeated.

13.2.8.2.3 If the results of the second background are unacceptable, the analyst should take steps to resolve the issue prior to any analysis.

13.2.8.3 For ATR, the background run prior to samples for each case serves as the blank for that case. This background/blank shall be printed and stored in the corresponding case file.

13.2.8.4 For solid samples, cover the center of the crystal with sample. Close and secure the bridge. Press the anvil against the sample by turning the anvil screw clockwise until it spins without further tightening.

13.2.8.5 For liquid samples, place a drop or two of liquid directly onto the ATR crystal. Use enough sample to cover the crystal completely. If the sample is volatile, place the cover over the sampling area to prevent evaporation during analysis. The bridge is not lowered during analysis.

13.2.8.6 If the sample requires an extraction, the sample in an organic solvent may be dropped on a crystal and allowed to evaporate to form a film. The bridge is not lowered during analysis. An o-ring may be used to contain the liquid as it is placed on the crystal.

13.3 Gas Phase FTIR via Gas Chromatography

13.3.1 The GC accessory provides FTIR data for samples in solution and vapor phases (head space analysis) using a continuous flow cell. The MCT-A detector scans from 4000 – 650 cm^{-1} and requires cooling with liquid nitrogen prior to analysis.

13.3.2 The spectra produced must be compared to known spectra taken under similar conditions.

13.3.3 GC-FTIR analysis is especially useful for phenethylamines due to the greater number of differences found in the gas phase fingerprint region versus the condensed phase fingerprint region. Also, the gas phase spectra often display more obvious differences between phenethylamines than their GC/MS spectra.

13.3.4 Procedure:

13.3.4.1 The instrument configuration should be set to Series, and the experiment set to GC-FTIR interface.

13.3.4.2 Parameters for the instrumentation are as follows:

- Split 5:1
- Column 30m x 0.32 mm i.d.
- Flow 1.5 – 8.0 mL/min.
- Resolution 8.0
- # of scans 8

- Background 128 scans

13.3.5 An amount of sample (1 – 4 µL) is injected.

13.3.6 After the series is reconstructed and the baseline is corrected, the sample spectrum will be compared to a known gas phase spectrum.

13.4 Acceptance Criteria

13.4.1 When using FTIR as the primary structural elucidation technique, the sample spectrum should compare favorably with a spectrum of a known standard in both its overall appearance and in the presence and location of the major peaks. Due caution should be exercised when using the similarity index generated by the library search algorithm.

13.4.2 When using FTIR to differentiate cocaine base from cocaine hydrochloride or another salt form where GC/MS has been previously performed, the areas of the spectrum which are different between cocaine base and cocaine hydrochloride should be clear. Other areas may have interfering peaks present that do not mask the "salt form" identity.

13.4.3 Data which supports the analyst's conclusion shall be printed and included in the case file.

13.4.3.1 At a minimum, the data shall include:

- FS Lab number and Item number
- Date
- Instrument name
- Sampling information (e.g., ATR, KBr pellet, pentane extract)
- Method parameters (e.g., # scans, resolution, sample gain, mirror velocity, aperture)

14 GAS CHROMATOGRAPHY/MASS SPECTROMETRY

14.1 Introduction

14.1.1 Gas Chromatography/Mass Spectrometry (GC/MS) is a specific method of identification for most drug substances. MS can not differentiate between optical isomers. A sample is passed through a gas chromatographic column, effecting a separation of the components of the sample. The individual compounds then move into the mass spectrometer source where they are bombarded by electrons, producing charged ions. The ions of interest are positively charged fragments of the original compound. The ions are then separated, through a mass filtering process, according to their mass-to-charge ratios (m/z) and then collected by a detector. In the detector, the ions are converted to a proportional electrical current. The data system records the magnitude of these electrical signals as a function of m/z and converts this information into a mass spectrum. The mass spectrum is a record of the different ions (m/z) and the relative numbers of each ion (abundance). These spectra are characteristic for individual compounds, giving specificity for most types of drug substances.

14.1.2 Depending on the structure of the molecule, the amount and type of fragmentation will vary. Due to this, some drugs do not exhibit a molecular ion using electron impact mass spectrometry. Examples include barbiturates, lorazepam and methylphenidate.

14.1.3 Confirmation of an unknown spectrum is done by direct comparison with a known or suitable reference spectrum or through use of interpretation methods. Positive mass spectral results may be recorded in the analytical notes by listing the drug identified. It is not required to record the analyst's disagreement with library search results on the data.

14.2 Procedure

14.2.1 Samples will be dissolved in a suitable solvent, preferably methanol.

14.2.2 The general concentration should be determined by TLC or GC before being run on the GC/MS. The usual amount of sample delivered to the ion source for good qualitative results should be 8 - 160 ng. This correlates to an approximate range of solution concentrations of 0.5 – 10 mg/mL, based on a typical 60:1 split ratio with a 1 μL injection volume. In any case, sufficient abundance of the total ion chromatogram peaks needs to be achieved in order to produce acceptable spectra, without overloading the chromatographic system.

14.2.3 For analysis of volatile organics, such as amyl nitrite, the headspace may be injected. An air blank must be run prior to headspace analysis.

14.2.4 Chromatographic conditions may be determined by the chemist from the GC/MS standards file.

14.2.5 The mass spectrum will be obtained in full scan mode using an appropriate scan range for the compounds to be analyzed.

14.2.6 At a minimum, a blank consisting of the solvent(s) used to dissolve the samples must be run on the GC/MS systems, when any of the following conditions are met:

- Before each analyst's series of sample runs whether manual or autosampler methods are utilized.
- No more than 10 samples can be run before another blank or standard/blank combination is required. A sample's position relative to the blank shall be documented. This may be accomplished by several methods, including consecutive data file numbering when using "windows macros".
- Whenever there is a change in the chromatographic conditions of the instrument. Changes include other methods being loaded (except those that merely change the injection volume or split ratio) or run between blank and sample.

- It is strongly suggested that a solvent blank be injected immediately prior to a sample known to be extremely weak.

- Additional blanks may be run at the examiner's discretion.

- The injection order when running samples with standards should be either "standard, blank, sample(s)" or "blank, sample(s), standard."

14.2.6.1 The solvent blank must be of at least as large an injection volume of the same solvent as the sample to be injected. The upper limit injection volume is normally 4 μL.

14.2.6.2 The solvent blank must be run at the same or lower split ratio as the sample. The solvent blank shall be run directly before samples which are run at a reduced split ratio (e.g., 20:1 when typical methods use 60:1).

14.2.6.3 Any significant peaks in blank chromatograms must be properly investigated and documented in the referenced case file.

14.2.6.3.1 If a controlled substance or related compound is present in any concentration, the blanks and associated samples should be re-run.

14.2.6.3.2 If an interfering substance is present, the blanks and associated samples should be re-run.

14.2.6.3.3 Blanks and associated samples should be replaced and re-sampled, respectively, prior to further analysis if the same extraneous peaks are still present.

14.2.7 Sequencing via autosampler should be utilized whenever practical.

14.2.8 Sequences and/or samples (including standards and blanks) shall be recorded in the instrument logbook.

14.2.8.1 Data files should not be overwritten.

14.2.8.2 Sequence files should not be overwritten unless additional data files have been added during the sequence run.

14.2.8.3 Sequences and sequence log files shall be archived along with data files as per ¶ 35.7.5.

14.2.9 Data which supports the analyst's conclusion shall be printed and included in the case file.

14.2.9.1 At a minimum, the data shall include:

- Data file name
- Date and time
- Instrument name
- Method name
- Sample name

- Barcode number (may be handwritten) and vial number, if applicable
- Integrated total ion chromatogram
- A background subtracted mass spectrum and normalized tabulation for peaks

14.2.10 It is permissible to use GC/MS integrated retention times for GC retention time data. Standards used in the comparison must be run within a 24 hour period of the sample.

14.2.11 Compare spectra to standard spectra run under the same conditions, in-house or reputable "library" spectra or published standard spectra to verify sample identification. Due caution should be exercised

when using the PBM similarity index generated by the library search algorithm. Spectra may also be identified through the use of interpretation methods in conjunction with data generated from additional testing with the approval of the Section Supervisor.

14.3 Data Interpretation and Acceptance Criteria

14.3.1 Integrated retention times for analytes are expected to agree with the standard within 2 seconds (± 2 sec.) or 0.033 minutes for this to be considered a positive GC result.

14.3.2 When barcodes are utilized for autosampler vial sample tracking, the barcode number should be printed on the data as well as documented in the case notes. If the barcode is not printed on the data during data analysis, it must be handwritten and initialed after checking the vial's tray location.

14.3.3 In order for a mass spectrum to be considered definitive, all major peaks must have associated ^{13}C isotope peaks present.

14.3.4 For compounds such as cocaine, heroin and LSD, a molecular ion peak with associated ^{13}C isotope peak must be present in order for the result to be considered definitive.

14.3.5 For compounds, such as methamphetamine, amphetamine and related compounds, it is imperative that the $[M-H]^+$ ion and its associated ^{13}C isotope peak/molecular ion be present in mass spectra in order for the result to be considered definitive. (e.g., methamphetamine must have a 148 and 149 *m/z* ion)

14.3.6 For compounds that do not exhibit a molecular ion, examples include methylphenidate and fentanyl, the mass spectrum, when used in combination with TLC, retention time data and other testing, is sufficient for identification.

14.3.7 Compounds such as barbiturates and some benzodiazepines should be derivatized to improve chromatographic performance or confirm the predicted molecular ion. Techniques of derivatization include silylation, alkylation and acetylation (see ¶ 10.3.13). AccuTOF-DART may be used to confirm the predicted molecular ion. Alternate ionization methods for mass spectrometry (e.g., positive or negative chemical ionization or LC/MS) can also be used via Instrument Support with the approval of the Section Supervisor and Chemistry Program Manager.

14.3.8 For compounds identified and reported, anomalous mass peaks occurring above the molecular ion must be explained with data documentation in the case file. This may be accomplished using the ion reconstruct function of the ChemStation software. Easily recognizable column/septum bleed peaks, 207, 221, 267, 281, 327, 341, 355, 385, 415 and 429 *m/z* (see ¶ 36.33), occurring above the molecular ion may be labeled as such on the spectrum without further data documentation.

14.3.9 The strength of the sample/sensitivity of the instrument can be enhanced in the following ways:

- Up to 4 µL of solution may be injected.
- The sample can be concentrated and placed into an autosampler vial insert.
- The split can be lowered to 10:1.for split methods
- Splitless methods may be employed for samples containing small amounts of drugs including, for example, residues, LSD and fentanyl.

14.3.9.1 If the spectrum still does not meet the criteria, it should be reported as "Insufficient for Identification".

14.3.10 Chromatographic and mass spectrometer conditions will be dependent on the currently used instrument and technology available. If there is any question as to either, consult with the primary operator of the instrument being utilized.

15 AccuTOF-DART MASS SPECTROMETRY

15.1 Introduction

15.1.1 Direct Analysis in Real Time (DART) is an atmospheric pressure ionization technique that can analyze solids, liquids and gases by placing the test material into a heated gas flowing through the sampling area. Ionization occurs from the surface of the sampling medium. Coupling of this ion source with an accurate mass time-of-flight mass spectrometer (AccuTOF) gives quick and simple analyses with little to no sample preparation.

15.1.2 While ionization can be done in both positive and negative mode, the large majority of drugs give usable spectra in positive ion mode. Ionization in positive ion mode is accomplished by charging a heated helium gas stream, forming metastable helium ions which react with ambient water vapor, producing hydronium ions which subsequently react with the sample molecules to induce ionization. The mechanism of positive and negative ion production with the DART is discussed by Cody, *et. al.* (See Reference 15.3.1)

15.1.3 In general, DART ionization produces spectra with a characteristic peak at the protonated or deprotonated molecule. These ions are measured at their exact mass in the AccuTOF mass spectrometer. Elemental composition calculations, based on empirical formulas, can be performed on these ions to determine whether they fall within a specified range, usually measured in millimass units (mmu), of a known compound. While accurate-mass spectra have an inherent specificity, full identification is difficult if the possibility of an isomer exists. To increase the specificity of the technique, the voltages on the orifice1 of the AccuTOF are varied, which can produce spectra with extensive fragmentation by in-source collision-induced dissociation (CID). This can be accomplished by utilizing the function switching mode of the AccuTOF operating software. Function switching allows for simultaneous collection of spectra at several different orifice1 voltages (See Reference 15.3.2). Higher orifice1 voltages generally result in more characteristic ions being produced. The combination of accurate mass measurement of the protonated molecule and characteristic CID fragmentation allows for the production of spectra that can be used as part of an identification scheme for drugs of abuse.

15.2 General Drug Screening Method

15.2.1 Instrumentation, Instrument Parameters and Materials

15.2.1.1 The DART ion source is coupled to a JEOL AccuTOF™ mass spectrometer (JMS-100LC) and operated in positive-ion mode. This system is controlled by "Mass Center" software.

15.2.1.2 Method parameters are listed in Table 1 below.

Table 1: Method Parameters for DART_POS_SWITCH_02 (General Screening Method – Positive Mode)

Parameter	Setting	Parameter	Setting
Ion Guide Peak Voltage	600V	Mass Range	66 – 600 Daltons (Da)
Orifice1 Voltage	Variable (20V, 30V, 60V, 90V)	Helium Flow Rate	2.5 L/min.
Spectrum Recording Interval	Every 0.25 seconds	Gas Heater Temperature	275 °C
Orifice2 Voltage	Approximately 5V	Discharge Electrode Needle	4000V
Ring Lens Voltage	Variable Approximately 3-6V	Electrode1	150V
Orifice1 Temperature	80 °C	Electrode2	250V

15.2.1.3 Internal mass calibration is accomplished using a dilute solution of polyethylene glycol (PEG) 600 (Chem. Service, West Chester, PA) in methanol.

15.2.1.4 Separate tune files are established where the only difference is in the orifice1 voltage.

15.2.1.5 Cleaned glass melting point tubes

15.2.1.5.1 It is necessary to clean the melting point tubes prior to use as a sampling device. The procedure below removes the majority of the dioctyladipate contaminant from the tubes. It does not, however, remove ALL of this contaminant from every tube. A more exhaustive cleaning method may need to be employed if this peak interferes with analyte peaks of interest.

15.2.1.5.2 Capillary Tube Cleaning Procedure:

- Remove tubes from plastic container and place into beaker, closed end down.
- Squirt acetone onto tubes while moving them around in the beaker to attempt to rinse as well as possible.
- Remove tubes and discard acetone.
- Shake tubes slightly to remove excess acetone.
- Place tubes back in beaker, closed end down.
- Squirt, vigorously, with methanol, moving tubes to attempt to squirt down all tubes.
- Remove tubes, discard methanol.
- Repeat methanol wash.
- Place tubes, closed end UP, in another beaker and place in the vacuum oven.
- Turn on vacuum oven and dry tubes until methanol is evaporated.
- Transfer tubes to another dry beaker, closed end down.
- Remove several tubes at a time and hold them in the HOT effluent of a heat gun for several seconds.
- Repeat previous step until all tubes have been "heat treated".
- Place tubes into a clean, screw-top vial until ready for use.

15.2.2 Procedure

15.2.2.1 Although samples may be run in any chemical state, it is recommended that powders, tablets and capsules be dissolved in a suitable solvent (e.g., methanol, methylene chloride, ammonia saturated chloroform).

15.2.2.2 In general, samples are run by dipping the closed end of the glass melting point tubes into the sample solution and then immediately inserting the tube into the DART gas stream for several seconds. Replicate samplings (2-3) are recommended within the data file to more fully represent spectra of the analytes of interest. Let the melting point tube cool briefly between samplings in order to achieve better consistency when sampling volatile solvents. Other sampling methods (e.g., solids, plant materials, gases, dried liquids) may be run after consultation with primary instrument operator.

15.2.2.3 Bring DART and AccuTOF out of standby mode, load DART_POS_O1_20V tune file and set the AccuTOF to "Operate". For more detailed instructions, see Reference 15.3.3.

15.2.2.4 PEG 600 calibrant solution shall be run within each data file. Replicate samplings (2-3) are recommended for proper internal mass calibration.

15.2.2.5 A mixture of cocaine, methamphetamine and nefazodone shall be run within each data file. The cocaine protonated molecule (304.1549 Da) will be used for drift compensation and the methamphetamine and nefazodone are positive controls.

15.2.2.6 After you are finished collecting data, turn the DART to standby to conserve helium.

15.2.2.7 Averaged, background subtracted, centroided spectra are used for all data reduction.

15.2.2.8 Mass calibration is performed utilizing the PEG600 spectra in the 30V function.

15.2.2.8.1 Maximum error should be less than 0.003 mmu and standard error should be less than 0.001.

15.2.2.8.2 The polynomial fit must be set to 3 or 4. A maximum of two data points may be removed to improve the calibration curve. Care should be taken not to remove data points at either end of the calibration table so as not to decrease the effective mass range of the data file.

15.2.2.8.3 Data files, which include calibration data, are archived. Overwriting the working calibration file is acceptable since they can be regenerated.

15.2.2.8.4 Internal mass calibration should be applied to all functions where data will be used.

15.2.2.9 Internal mass drift compensation is performed using the cocaine spectrum in the 30V function.

15.2.2.9.1 Overwriting the working calibration file is acceptable since they can be regenerated.

15.2.2.9.2 Internal drift compensation results should be applied to all functions where data will be used.

15.2.2.10 After performing PEG600 internal mass calibration and internal mass drift compensation for cocaine, the protonated molecules (30V function) of methamphetamine and nefazodone shall be within ± 5 mmu of their calculated values of 150.1283 Da and 470.2323 Da, respectively.

15.2.2.10.1 The spectrum demonstrating that the cocaine, methamphetamine and nefazodone protonated molecules are within the above acceptance criterion shall be included in the case file.

15.2.2.11 Data processing

Spectra should be saved in JEOL-DX format for use by other data reduction software (e.g., SearchFromList, Elemental Composition Workshop). Repeat as necessary for spectra from other orifice1 functions.

15.2.2.12 Return AccuTOF and DART to standby mode. Do not turn the DART gas off before the temperature is down.

15.2.3 Data Interpretation

15.2.3.1 Data from the 20V function is used to obtain information regarding molecular weight and should be searched against the Drug Neutral Mass library.

15.2.3.2 Data from functions with higher Orifice1 values result in greater fragmentation, and should be searched against the single component standard and/or the drug preparation libraries for those voltages. Poly-drug spectra can lead to complex spectral interpretation. Care must be taken to evaluate search results using both types of information.

15.2.3.3 Comparison of spectra to in-house library search results should be included in the case file.

15.2.3.4 To report the identity of a drug indicated by this screening method, confirmation utilizing the normal analytical scheme is required.

15.3 Negative Screening Method including GHB

15.3.1 The Negative Screening Method must be run in consultation with the DART operator or designated backup.

15.3.2 Instrumentation, Instrument Parameters and Materials

15.3.2.1 The DART ion source is coupled to a JEOL AccuTOF™ mass spectrometer (JMS-100LC) and operated in negative-ion mode. This system is controlled by "Mass Center" software.

15.3.2.2 Method parameters: Use the NEG_GHB.inf file for DART parameters (helium 2.5 L/min @ 300 deg C).

15.3.2.2.1 After changing AccuTOF to ESI-mode, load the "DART_-GHB" tune file or load the latest negative ion tune file. Ensure the "ion guide peaks voltage" setting is at 600V and the "Orifice 1 Voltage" is set to -20V. When running a sample, specify MS acquisition method and set the scan mass range to 65-300 Da.

15.3.2.3 Internal mass calibration solution: polyethylene glycol (PEG) 600 (Chem. Service, West Chester, PA) in methanol.

15.3.2.4 QC check solution: malic acid (calculated mass: 133.0137 Da) in methanol.

15.3.2.5 GHB (calculated mass: 103.0395 Da) solution in methanol.

15.3.3 Procedure

15.3.3.1 Although samples may be run in any chemical state, it is recommended that powders, tablets and capsules be dissolved in a suitable solvent (e.g., methanol, methylene chloride, ammonia saturated chloroform). Liquid samples may be run whole or diluted (with DI water) if too viscous.

15.3.3.2 Data files should contain PEG600 calibration, malic acid QC check, GHB standard and samples.

15.3.3.3 For data reduction, choose "DART_PEG_NEG" for the PEG600 calibration peak table. In SearchFromList, choose "subtract" for the charge carrier.

15.3.3.4 If the sample is a drink, refer to the Bennett article to determine administrative cutoffs for various drink matrices and the reasoning behind these.

15.3.3.5 For samples that turn out to be negative, and if sample size permits, spike a milliliter of sample with one milligram of GHB standard and run on the AccuTOF-DART to demonstrate that if GHB were present in the sample, it would have produced a spectrum under the conditions used for that matrix.

15.4 References

15.4.1 Cody RB, Laramee JA, Nilles JM, Durst HD. "Direct Analysis in Real Time (DART) mass spectrometry" *JEOL News* 2005; 40(1): 8-12.

15.4.2 JEOL, Inc. Application note: *LC/MS: Identification of unknowns by combining exact mass measurement with the NIST 02 Mass Spectral Database Similarity Search*, www.JEOL.com/ms/accutof.html, January 2003.

15.4.3 221-D101 DFS AccuTOF-DART Operating Instructions.

15.4.4 Bennett MJ and Steiner RR, "Detection of Gamma-Hydroxybutyric Acid in Various Drink Matrices via AccuTOF-DART", *J Forensic Sci*, 2009, 54(2), 370-5.

15.4.5 Steiner RR, Larson RL. "Validation of the Direct Analysis in Real Time source for use in forensic drug screening." *J Forensic Sci* 2009; 54(3):617-22.

16 GENERAL ANALYTICAL METHODOLOGY

16.1 Introduction

The following sections include the general methodology for drug groups and drug compounds. At various times, a drug chemist will encounter drug substances for analysis which do not fall under the following categories or that require specialized analysis. For these cases a general guideline for analysis can be followed based on the general unknown/powder flow chart. It should be noted that sample size or other circumstances may require a rearrangement or modification of one or more steps. These modifications must be documented in the case file.

16.2 Techniques

16.2.1 Positive color test reactions are denoted in each drug section.

16.2.2 Recommended extraction solvents and procedures are listed in each drug section. For compounds not individually listed, extraction and solubility information are found in references such as *Clarke's Isolation and Identification of Drugs* and the *Merck Index*. Extraction solvents used in case work will be recorded in the case notes. A procedure blank shall be run for multi-step extractions and documented in the case notes.

16.2.3 Unless otherwise noted in the following sections, the chemist should consult the MS file for GC/MS method conditions and the GC file for GC method conditions.

16.2.4 All efforts should be made to utilize the automated data acquisition and reduction functions on the mass spectrometers and other instruments.

16.2.5 In the presence of controlled substances, minor or inconsequential GC peaks or TLC spots may, at the examiner's discretion, be ignored (e.g., cis- and/or trans-cinnamoylcocaine in the presence of cocaine).

16.3 Reference Collections

16.3.1 Reference collections of data or materials used for identification, comparison or interpretation shall be fully documented, uniquely identified and properly controlled.

16.3.2 Purchased data libraries (reference collections) are fully documented and uniquely identified. No changes may be made to purchased reference collections. Examples of such libraries include:

- GC/MS NIST
- GC/MS Wiley
- FTIR Aldrich
- FTIR Georgia State ATR
- FTIR Thermo/Nicolet White Powders
- FTIR Thermo/Nicolet Chemical Warfare Agents
- FTIR Georgia State Crime Lab
- FTIR Georgia Forensic Sample Library
- FTIR DEA Full 4cm-1 resolution KBr collection
- FTIR DEA Full 4 cm-1 resolution KBr Liquids

16.3.3 Data libraries (reference collections) obtained from reputable forensic sources are fully documented and uniquely identified. No changes may be made to these reference collections. The addition or removal of forensic libraries must be approved by the Chemistry Program Manager. Current forensic libraries approved for use:

- GC/MS AAFS
- GC/MS ENFSI
- GC/MS TIAFT

- Synthetic Cannabinoid MS Library (syncann)
- SWGDRUG Mass Spectral Library
- FTIR Durascope ATR (NCIS)
- FTIR Mills (Georgia)

16.3.4 In-house data libraries include:

- GC/MS VAL
- FTIR DFS ATR
- FTIR NOVA DRUGS ATR
- FTIR VCU General Chemicals
- AccuTOF-DART Drug Standard Library (_ori20, _ori30, _ori60 and _ori90)
- AccuTOF-DART Prep Library (_ori20, _ori30, _ori60 and _ori90)

16.3.5 At a minimum the following information shall be included with each new entry into in-house data libraries:

- Compound name
- Pharmaceutical preparation or drug standard identifier
- Date
- Initials of person entering data

16.3.6 For in-house libraries, each entry is automatically identified by a unique tracking number generated by the instrument software.

16.3.7 In-house libraries shall be generated or modified either by an instrument operator or by a designee of the Section Supervisor.

16.3.8 A reference collection of pharmaceutical preparations and drug standards is maintained for use as reference standards. Receipt and use of these standards is recorded and maintained as required by § 54.1-3404. These standards are uniquely identified through the use of laboratory lot/tracking numbers in addition to the manufacturer's lot number. Access to drug standards and records is limited to section members.

17 STIMULANT METHODOLOGY

17.1 Brief Pharmacology: Central nervous system stimulants and appetite suppressants which are commonly referred to as "uppers".

17.2 Drug Group Examples: Amphetamine, methamphetamine, phentermine, phendimetrazine, phenmetrazine, methcathinone and methylphenidate.

17.3 Types of Samples

17.3.1 Many stimulants are found in pharmaceutical preparations.

17.3.2 Methamphetamine, in particular, is often clandestinely manufactured.

17.4 Scheduling

- Schedule I Methcathinone
- Schedule II Amphetamine, methamphetamine, phenmetrazine and methylphenidate
- Schedule III Phendimetrazine
- Schedule IV Phentermine
- Non-scheduled, but listed in Code of Virginia §18.2-248(K) as "methamphetamine precursor drugs" – ephedrine, pseudoephedrine and phenylpropanolamine

17.5 Extraction

17.5.1 May be extracted from basic aqueous solutions with organic solvents. This is routinely necessary to obtain good chromatographic results with the phenethylamine-type compounds. A procedure blank shall be run for multi-step extractions and documented in the case notes.

17.5.2 May be dry extracted with methanol or other organic solvents.

17.6 Color Test Results

17.6.1 Marquis Results

- Most phenethylamines - Orange → Brown
- Phentermine, phenmetrazine and phendimetrazine - do not give an orange color
- Add water to the well after noting color results and place under longwave UV. Methamphetamine fluoresces blue while MDMA will not.

17.6.2 Nitroprusside (Fiegel's Test) Results

- Secondary amines - dark blue

17.6.3 TBPEE Results

- Primary amines - purple
- Secondary amines - blue
- Tertiary amines - red

17.7 TLC

17.7.1 Extraction of the sample may be necessary to get good TLC results.

17.7.2 Baths

- TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.
- TLC9 separates methamphetamine from MDMA.
- TLC13 separates ephedrine from pseudoephedrine. The TLC13 bath shall be used to identify either ephedrine or pseudoephedrine alone.

17.7.3 Detection sprays

17.7.3.1 Fluorescamine (Fluram) for primary amines.

17.7.3.2 Iodoplatinate for secondary and tertiary amines. Iodoplatinate results may be enhanced by overspraying with ceric sulfate.

17.7.3.3 Dragendorff

17.7.3.4 Ninhydrin is recommended for ephedrine and pseudoephedrine.

17.8 UV

17.8.1 Extraction of the sample may be necessary to get a good UV spectrum.

17.8.2 Results: Phenethylamine-type compounds give a triplet benzenoid spectrum with associated minima. (e.g., amphetamine - max. 251.5, 257, and 263 nm)

17.9 GC

17.9.1 Extraction of the sample may be necessary to obtain good chromatography.

17.9.2 Acetyl Derivative, to improve chromatographic performance, if necessary: The acetyl derivative of phenethylamines is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. The acetyl derivative should have a longer retention time than the underivatized compound and may require a higher chromatographic temperature than the underivatized compound.

17.10 GC/MS

17.10.1 The concentration of the sample must be strong enough to detect the $[M-H]^+$ ion and its associated ^{13}C isotope peak/molecular in order for the result to be considered definitive. (e.g., methamphetamine must have a 148 and 149 *m/z* ion)

17.10.2 Ephedrine and pseudoephedrine can not be differentiated by their mass spectra. In the absence of the 166 ion, an acetyl derivative or AccuTOF DART is required for identification.

17.11 FTIR

17.11.1 Extraction from excipients may be necessary to obtain a good spectrum or chromatographic performance.

17.11.2 GC-FTIR is a useful technique to differentiate between phenethylamine-type compounds.

17.12 Reporting

17.12.1 See ¶ 32.14 for reporting of listed substances.

17.13 Amphetamine/Methamphetamine Quantitation

17.13.1 See GC ¶ 10 for general quantitation procedure. Amphetamine and methamphetamine will most often be calculated and reported as the base.

17.13.2 Materials

- Methylene Chloride or Chloroform
- Amphetamine Sulfate
- Methamphetamine HCl
- Tridecane
- 4N NaOH solution
- Class A volumetric flasks
- Class A volumetric pipettes
- Analytical balance

17.13.3 Internal Standard Solution

17.13.3.1 Prepare a sufficient volume to dilute the standard solutions and all samples.

17.13.3.2 Prepare a 1 mg/mL solution of tridecane in methylene chloride or chloroform in the appropriate volumetric flask.

17.13.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

17.13.4 Standard Solutions

17.13.4.1 Prepare a 2 mg/mL standard

- Weigh approximately 20 mg of the desired standard
- Dilute to approximately 5 mL with D.I. or R.O. water
- Make basic with 4N sodium hydroxide until pH is above 10
- Use a volumetric pipette to deliver the internal standard solution
- Extract two times with 5 mL portions of internal standard solution
- Combine the extracts for further analysis

17.13.4.2 Prepare a solution of another concentration within the linear range to use as the check standard. Weigh at least 10 mg of standard and combine the extracts from two extractions as outlined above.

17.13.4.3 Mathematical Conversion:

Unless amphetamine sulfate or methamphetamine hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated.

To convert amphetamine sulfate to free base, multiply the weight amount of amphetamine sulfate by 0.7338 (270.42 F.B./368.48 SO_4). Amphetamine sulfate is dibasic. This will give the free base weight of amphetamine in the standard solution.

To convert methamphetamine HCl to free base, multiply the weight amount of methamphetamine HCl by 0.8037 (149.24 F.B./185.70 HCl). This will give the free base weight of methamphetamine in the standard solution.

17.13.5 Sample Solution

Prepare two separate sample solutions for a duplicate analysis. For each, weigh approximately 20 mg (or more) of the sample, dilute to 5 mL with water and extract as with the standard solution. Run a duplicate analysis by preparing two separate solutions.

17.13.6 GC parameters

- Column: 15 m HP-1 capillary (0.25 mm i.d, 0.25 µm film thickness)
- Oven temperature: approximately 70 - 210°C at 30°C per minute
- FID temperature : 280°C

17.13.7 Linear Range

17.13.7.1 The validated linear range of both the amphetamine and methamphetamine method is 0.5 – 5 mg/mL.

17.13.7.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

17.13.8 Amphetamine elutes prior to Methamphetamine which elutes prior to tridecane.

17.13.9 Calculations, Acceptance Criteria and Reporting

See ¶ 10.4.4.7 – 10.4.4.10

17.14 Differentiation of the Stereoisomers of Methamphetamine using GC Derivatization (Determination of "ICE")

17.14.1 "Ice" is a crystalline form of nearly pure *d*-methamphetamine.

17.14.2 Isomer determination is not required for normal analysis, but may be requested by an agency to provide information as to the manufacturing process.

17.14.3 Procedure

17.14.3.1 Samples of methamphetamine should be dissolved in $CHCl_3$ or CH_2Cl_2 for GC analysis. Extraction is not usually necessary.

17.14.3.2 Standards, consisting of *d*- or *l*- (optically pure) methamphetamine and the *d,l*-racemate should be prepared in $CHCl_3$ or CH_2Cl_2 at concentrations of approximately 1-2 mg/mL. It is not necessary to use both optically pure isomer standards.

17.14.3.3 n-Trifluoroacetyl-l-prolylchloride (*l*-TPC) may be obtained from Regis Chemical Co. (Chicago, Il.) or Sigma/Aldrich. *l*-TPC is supplied as 0.1M in $CHCl_3$ with 1-2% of the *d* isomer (*d*-TPC).

17.14.3.4 GC parameters:

- Injection port: 270° C
- Detector: 280° C;
- Oven: 215° C isothermal
- Split flow: approx 100:1 (standard split liner)
- Columns:

- HP-1 (Methyl silicone) 0.25 mm x 15 m x 0.25 μm (i.d. x length x film thickness)
- HP-5 (5% Phenyl methyl silicone) 0.25 mm x 15 m x 0.25 μm
- Carrier gas: helium

17.14.3.5 Both the optically pure and the racemate standards need to be injected. The racemate will check the resolution of the chromatographic system and the optically pure standard will determine the peak of interest. Baseline resolution should occur with the racemate/ƒ-TPC derivatives.

17.14.3.6 Load a 10 μL syringe with 1.0 μL ƒ-TPC, 0.5 μL air and 1.0 μL methamphetamine solution (sample or std). Inject directly into the GC.

17.14.3.7 On both columns, the ƒ- methamphetamine / ƒ-TPC derivative elutes first.

17.14.3.8 Several additional peaks may be seen in the chromatogram. One such peak, occurring at a retention time approximately one minute prior to the ƒ- methamphetamine / ƒ-TPC peaks, is due to excess ƒ-TPC. As methamphetamine concentration increases, this peak will decrease in height. Other peaks, very close to the solvent front, appear to be due to decomposition of the ƒ-TPC reagent.

17.14.4 Methamphetamine isomers are not to be routinely reported on the certificate of analysis.

17.14.5 References

17.14.5.1 Fitzgerald, R.L., et. al., "Resolution of Methamphetamine Stereoisomers in Urine Drug Testing: Urinary Excretion of R(-)-Methamphetamine Following use of Nasal Inhalers", *J. Anal. Tox.*, Vol 12, Sept/Oct 1988, pp. 255-259.

17.14.5.2 Fitzgerald, R.L., et. al., "Determination of 3,4-Methylenedioxyamphetamine and 3,4-Methylenedioxymethamphetamine Enantiomers in Whole Blood", *J. Chromatogr.*, 490 (1989), pp. 59-69.

18 COCAINE AND LOCAL ANESTHETIC METHODOLOGY

18.1 Brief Pharmacology: Depresses sensation of pain, may cause CNS stimulation producing excitement and erratic behavior.

18.2 Drug Group Examples: Cocaine, procaine, benzocaine, tetracaine, lidocaine, as well as the isomers of cocaine such as pseudococaine, allococaine, pseudoallococaine.

18.3 Scheduling

- Schedule II – cocaine
- Schedule VI or non-controlled, depending on their packaging – procaine, lidocaine, benzocaine and tetracaine

18.4 Extraction

18.4.1 May be extracted from basic aqueous solutions with organic solvents. A procedure blank shall be run for multi-step extractions and documented in the case notes.

18.4.2 May be dry extracted with methanol or other organic solvents.

18.5 Color Test Results

18.5.1 $Co(SCN)_2$ Results

- Cocaine HCl, lidocaine, procaine, tetracaine, benzocaine – blue precipitate
- PCP, heroin and other compounds, including flour – weak blue

18.5.2 Acid Modification to $Co(SCN)_2$ Results

18.5.2.1 This test may also aid in distinguishing cocaine base.

- Cocaine base + $Co(SCN)_2$ – no reaction
- Upon the addition of the HCl, a blue precipitate readily forms and remains.

18.5.3 $SnCl_2$ Modification to $Co(SCN)_2$ Results

18.5.3.1 This test can help to distinguish between some "caines".

- Cocaine salt + $Co(SCN)_2$ – blue precipitate forms
- If $SnCl_2$ is added to the spot well, the blue color remains in the presence of cocaine salt, but the blue color will fade with some other "caines".

18.5.3.2 This test may also aid in distinguishing cocaine base.

- Cocaine base + $Co(SCN)_2$ – no reaction
- Upon the addition of the $SnCl_2$ reagent which contains HCl, a blue precipitate readily forms and remains.

18.5.4 Scott's Modification of Ruybal's test for Cocaine Results

18.5.4.1 May get false positive with lidocaine and diethylpropion.

18.5.4.2 Sample is placed in $Co(SCN)_2$ solution to give blue precipitate. Concentrated HC1 is added (1 drop) to make the precipitate disappear and give a pink solution. $CHCl_3$ is added, and the mixture is shaken. The $CHCl_3$ layer turns blue in the presence of cocaine.

18.5.5 Bate's Modification to $Co(SCN)_2$ Results

18.5.5.1 This test may aid in distinguishing cocaine base from its salts.

- Cocaine base + $Co(SCN)_2$ – No Reaction
- Upon the addition of Marquis reagent, a blue precipitate readily forms and remains.

18.6 TLC

18.6.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

- PCP migrates similar to cocaine if the baths are not fresh or if samples are very concentrated.
- Cocaine and tetracaine separate if baths are fresh.
- Lidocaine migrates close to cocaine in TLC2 (18:1) but not in TLC1 (9:1).

18.6.2 Detection sprays:

18.6.2.1 Iodoplatinate, results may be enhanced by overspraying with ceric sulfate.

18.6.2.2 Ehrlich's: Procaine and benzocaine are yellow if the plate is oversprayed.

18.7 UV

18.7.1 Cocaine – maximum at 232 nm in acid

18.8 FTIR

18.8.1 FTIR is the most easily performed and definitive method for distinguishing cocaine base from its salts.

18.8.2 Base determinations will be routinely performed in the following types of cases:

- The weight of cocaine is over 250 grams
- The officer has requested cocaine base analysis for possible federal prosecution
- Task Force or Interdiction cases, when required
- Cases from certain jurisdictions involving firearms

18.8.3 Sample preparation

- KBr pellet
- ATR

18.8.4 Dry extraction with high purity n-pentane or n-hexane will distinguish cocaine base from its salts.

18.8.5 Further extractions based on solubility differences between the cocaine and excipients may be required.

18.8.6 Reporting

18.8.6.1 Materials containing cocaine base (including mixtures of cocaine base and cocaine hydrochloride) will be reported as "Cocaine base."

18.8.6.2 Materials containing cocaine hydrochloride (unless mixed with cocaine base) will be reported as "Cocaine Hydrochloride."

18.9 Cocaine Quantitation

18.9.1 See GC ¶ 10 for general quantitation procedure.

18.9.2 Materials

- Methylene Chloride or Chloroform
- Dicyclohexylphthalate (DCHP)
- Cocaine HCl
- Class A volumetric flasks
- Analytical balance

18.9.3 Internal Standard Solution

18.9.3.1 Prepare a sufficient volume to dilute the cocaine standard solutions and all samples.

18.9.3.2 Prepare a 1.5 - 2 mg/mL solution of DCHP in methylene chloride or chloroform in the appropriate volumetric flask.

18.9.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

18.9.4 Cocaine Standard Solutions

18.9.4.1 Weigh at least 10 mg of cocaine HCl and quantitatively transfer to a 10 mL volumetric flask with internal standard solution. Fill to mark with internal standard solution. This results in a solution of 1 mg/mL cocaine HCl in internal standard solution.

18.9.4.2 Prepare a solution of another concentration within the linear range to use as the check standard. Weigh at least 10 mg of cocaine HCl and quantitatively transfer to a volumetric flask. Fill to mark with internal standard solution.

18.9.5 Standard Salt Form Conversion

Unless cocaine hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of cocaine HCl to free base, multiply the concentration (mg/mL) of cocaine HCl by 0.8929 (303.4 F.B./339.8 HCl). This will give the concentration of free base in the standard solution.

18.9.6 Sample Preparation

Prepare two separate sample solutions. For each, weigh approximately 20 mg of sample into a 10 mL volumetric flask. Dilute with internal standard to the mark.

18.9.7 GC parameters

- Column: 15 m HP-1 (0.25 mm i.d. 0.25 µm film thickness)
- Oven temperature: approximately 220 – 245 °C
- FID temperature : 280°C

18.9.8 Linear Range

18.9.8.1 The validated linear range of the cocaine method is 0.5 – 5 mg/mL.

18.9.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

18.9.9 Cocaine elutes prior to DCHP.

18.9.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 10.4.4.7 - 10.4.4.10

19 BARBITURATE METHODOLOGY

19.1 Brief Pharmacology: Central nervous system depressants commonly known as "Downers"

19.2 Drug Group Examples: Butalbital, pentobarbital, secobarbital, allobarbital, amobarbital, butabarbital, barbital, and phenobarbital

19.3 Types of Samples

Most barbiturates are found in pharmaceutical preparations.

19.4 Scheduling

- Schedule II — Amobarbital, secobarbital, and pentobarbital
- Schedule III — Most barbiturates
- Schedule IV — Phenobarbital
- Schedule VI or non-controlled — Some preparations of phenobarbital, butalbital, and other such barbiturates are specifically exempted from control.

- Appropriate caution must be exercised when determining their control status. Any questions should be answered by consulting appropriate compendia references such as the PDR, Poison Control, DEA Logo Index and the appropriate state or federal codes, as well as, informing the Section Supervisor. If any question remains, DO NOT include the schedule in your report.
- Code of Virginia - §54.1-3445 – 54.1-3455 – Searchable using the online Legislative Information System (http://leg1.state.va.us/000/src.htm)
- Federal Controlled Substances Schedules - www.deadiversion.usdoj.gov/schedules/index.html#list

19.5 Extraction

Barbiturates may be extracted from either acidic or weak basic aqueous solutions with organic solvents. A procedure blank shall be run for multi-step extractions and documented in the case notes.

19.6 Color Test Results

19.6.1 Dille-Koppanyi - This is a two part test. Place 2 drops of DK1 reagent in a well. Add sample. Add 1 drop of DK2 reagent. When doing multiple samples, they should be separated to avoid cross-contamination due to reagent spreading. Barbiturates give a purple color. False positives from: glutethimide, theophylline and hydantoins.

19.6.2 $Co(SCN)_2$ - faint blue on barbiturates with an unsaturated side chain (i.e., butalbital).

19.6.3 Parri - blue

19.7 TLC

19.7.1 Baths: The isopropyl ether bath (TLC7) will separate most of the barbiturates from one another.

19.7.2 Detection sprays

19.7.2.1 $KMnO_4$ reacts with barbiturates with an unsaturated side chain to yield a yellow spot on a purple background.

19.7.2.2 $HgSO_4$ - spray very heavily to give light spots on an off-white background.

19.7.2.3 Diphenylcarbazone - overspray for $HgSO_4$ gives pink spots for barbiturates.

19.8 UV

19.8.1 Extraction of the sample may be necessary to get a good spectrum.

19.8.2 Barbiturates should be run in both acidic and basic media due to their characteristic bathochromic shift as the solution becomes basic.

19.9 GC

19.9.1 Extraction or derivatization of the sample may be necessary to get good chromatographic peak shape.

19.9.2 Alkyl Derivative: trimethylanilinium hydroxide (TMAH)

19.9.2.1 See GC section 10 for procedure.

19.9.2.2 Formation of the methyl derivative will generally decrease the retention time significantly.

19.10 GC/MS

19.10.1 Barbiturates most often do not exhibit a molecular ion peak and require derivatization.

19.11 FTIR

19.11.1 Extraction may be necessary to obtain a useful FTIR spectrum.

20 NARCOTIC METHODOLOGY

20.1 Brief Pharmacology: Analgesic, sedative effects and causes constipation.

20.2 Drug Group Examples: Morphine, heroin, hydromorphone, pentazocine, codeine, hydrocodone, oxycodone, methadone, propoxyphene, pethidine (meperidine), and fentanyl

20.3 Scheduling

- Schedule I — Heroin
- Schedule II — Morphine, oxycodone, hydromorphone, methadone, pethidine (meperidine), fentanyl, codeine (pure), hydrocodone (pure), levomethorphan, 6-monoacetylmorphine (morphine derivative)
- Schedule III — Some preparations of hydrocodone or codeine
- Schedule IV — Pentazocine
- Schedule V — Some preparations of codeine (usually cough preparations)
- Non-controlled — Dextromethorphan

- Appropriate caution must be exercised when determining the control status of compounds listed in multiple schedules. Any questions should be answered by consulting appropriate compendia references such as the PDR, Poison Control, DEA Logo Index and the appropriate state or federal codes. Marked capsules or tablets need not be quantitated. Questionable samples require at least a semi-quantitative workup to determine the schedule.

20.4 Extraction

20.4.1 Most narcotics may be extracted from basic solutions with organic solvents. A procedure blank shall be run for multi-step extractions and documented in the case notes.

20.4.2 Morphine may be extracted from aqueous solution by the addition of a carbonate/bicarbonate buffer and extracting with $CHCl_3$ or $CHCl_3$/isopropanol (8:2). Morphine sulfate is not soluble in chloroform.

20.4.3 Methadone is often found in orange juice or Tang-type orange drink. The solution should be made basic with sodium carbonate and extracted into $CHCl_3$ or CH_2Cl_2.

20.4.4 Narcotics in cough syrups may be extracted from basic solutions with organic solvents.

20.5 Color Test Results

Most narcotics give colors with Marquis, Meckes and Froehdes reagents (see Table 2). Numerous other materials give similar colors, such as methapyrilene, glycerol guaiacolate (guaifenesin), and pyrilamine, and are also included.

TABLE 2: Positive Color Test Reactions

Compound	Marquis	Meckes	Froehdes	HNO_3
Morphine	Purple	Green	Purple	Red
Heroin	Purple	Green	Purple	Yellow
Codeine	Purple	Blue-green	Green	Yellow
Propoxyphene	Black	Orange/brown	Brown	No reaction
Meperidine	Orange	Yellow-green	Grey	
Pentazocine	Red→olive green	Olive green	Blue	Yellow
Hydromorphone	Yellow→red	Yellow-orange	Blue→purple	Yellow-orange
Hydrocodone	Purple	Green	Lt. yellow	No reaction
Oxycodone	Yellow→purple	Yellow→olive	Yellow	No reaction

Compound	Marquis	Meckes	Froehdes	HNO_3
Methadone	Slow pink	Yellow-green→green		
Methapyrilene	Purple	Purple	Purple	No reaction
Guaifenesin	Reddish purple	Green/purple	Green with purple streaks	Yellow
Pyrilamine	Purple	Purple	Purple	

20.6 TLC

20.6.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

20.6.2 Detection sprays:

20.6.2.1 Iodoplatinate

20.6.2.2 Ceric Sulfate - Fentanyl may require the use of ceric sulfate as an overspray due to the minute amounts of this material found in most pharmaceutical preparations.

20.7 UV

20.7.1 Not usually unique to the specific compound.

20.7.2 Results:

- Morphine – max. at 285 nm in acid
- Heroin – max. at 280 nm in acid (Heroin will often hydrolyze to monoacetylmorphine and morphine with prolonged exposure to aqueous solutions.)
- Methadone – max. at 250 and 292 nm in acid
- Pethidine (Meperidine) – triplet - max. at 258 nm in acid

20.8 Dextromethorphan Enantiomer Determination

20.8.1 Because of the scheduling differences between levomethorphan (Schedule II) and dextromethorphan (non-controlled), a microcrystal test must be performed for differentiation unless found in a recognizable pharmaceutical preparation.

20.8.2 Materials:

- Glass slides and coverslips
- Polarizing microscope
- 10% Platinic Chloride solution in water (w/v)
- 1% acetic acid solution in water
- Dextromethorphan reference standard

20.8.3 Procedure

20.8.3.1 As a negative control/blank, place one drop of 1% acetic acid in water on a glass slide. Then add a portion of a drop of 10% platinum chloride in water and cover. No crystals should form within 2 minutes.

20.8.3.1.1 If crystals form within 2 minutes, discard slide and cover slip, if used, and repeat.

20.8.3.1.2 If the results of the second blank are unacceptable, the analyst should take steps to resolve the issue (e.g., remaking reagents) prior to any analysis.

20.8.3.2 Place one drop of 1% acetic acid in water to a small portion (less than 1mg of pure dextromethorphan is needed) of the dextromethorphan standard on a glass slide. Then add a portion of a drop of 10% platinum chloride in water and cover. Feathery dendrites will form within 2 minutes.

20.8.3.3 Place one drop of 1% acetic acid in water to a small portion of the sample on a glass slide. Then add a portion of a drop of 10% platinum chloride in water and cover. Feathery dendrites will form when the sample is a pure enantiomer. A racemic mixture will not form these microcrystals.

20.8.3.4 To verify the identity as the dextro isomer, mix a small amount of sample with approximately the same amount of dextromethorphan reference standard on a glass slide. Add the acetic acid and platinum chloride solutions as stated above. Observe any microcrystalline formation. If the sample is dextromethorphan, the feathery dendrites will form; if the sample is levomethorphan or racemethorphan, no dendrite-shaped crystals will form.

20.8.3.5 The microcrystal formations should be contemporaneously verified or photographed. Verification must be documented in the case notes with the initials of the verifier and the date.

20.8.3.6 The sample may need to be purified to allow for crystal formation. Mixtures of dextromethorphan and MDMA will need to be separated before the crystal test, as pure MDMA reacts with platinum chloride to form similar, but not the same, microcrystals.

20.8.3.7 Reference: Fulton, Charles C. *Modern Microcrystal Tests for Drugs* , New York: Wiley-Interscience, 1969, pp. 58-59.

20.9 Heroin Quantitation

20.9.1 See GC ¶ 10 for general quantitation procedure.

20.9.2 Reagents:

- Methylene Chloride or Chloroform
- Heroin HCl or Heroin HCl monohydrate Standard
- Dicyclohexylphthalate (DCHP)
- Class A volumetric pipettes
- Analytical balance

20.9.3 Internal Standard Solution:

20.9.3.1 Prepare a sufficient volume to dilute the heroin standard solution and all samples.

20.9.3.2 Prepare a 1.5 - 2 mg/mL solution of DCHP in methylene chloride or chloroform in an appropriate volumetric flask.

20.9.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

20.9.4 Heroin Standard Solutions:

20.9.4.1 Weigh at least 10 mg of Heroin HCl and quantitatively transfer to a 10 mL volumetric flask with internal standard solution. Fill to mark with internal standard solution. This results in a solution of ~1.0 mg/mL Heroin HCl in internal standard solution.

20.9.4.2 Prepare a solution of another concentration within the linear range to use as the check standard. Weigh at least 10 mg of heroin HCl and quantitatively transfer to volumetric flask. Fill to mark with internal standard solution.

20.9.5 Standard Salt Form Conversion

Unless heroin hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of heroin hydrochloride monohydrate to free base, multiply the concentration (mg/mL) of heroin HCl by 0.8714 (369.4 F.B./423.9 salt). This will give the concentration of free base in the standard solution.

20.9.6 Sample Preparation

20.9.6.1 Prepare two separate sample solutions. For each, weigh 10-40 mg of sample into a volumetric flask (Class A) of an appropriate volume. Dilute with internal standard to the mark. For example, 30 mg of a 20% heroin sample in 5 mL internal standard solution results in a 1.2 mg/mL solution.

20.9.7 GC parameters:

- Column: 15 m HP-1 capillary (0.25 mm i.d., 0.25 µm film thickness)
- Oven temperature: approximately 260-280° C
- FID temperature: 280° C

20.9.8 Linear Range

20.9.8.1 The validated linear range of the heroin method is 0.5 – 5 mg/mL.

20.9.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

20.9.9 DCHP elutes prior to Heroin. Peaks between DCHP and Heroin are often due to monoacetylmorphine or acetylcodeine.

20.9.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 10.4.4.7 - 10.4.4.10

21 PHENCYCLIDINE (PCP) AND ANALOG METHODOLOGY

21.1 Brief Pharmacology: PCP is classified as a dissociative anesthetic. PCP is used as an animal tranquilizer. It is sometimes called angel dust, crystal, or hog, and known as boat or loveboat when placed on marijuana.

21.2 Scheduling

- Schedule I - TCP - thienylcyclohexylpiperidine (thiophene analog of PCP)
- Schedule I - PHP - 1-(1-phenylcyclohexyl) pyrrolidine (pyrrolidine analog of PCP)
- Schedule I - PPP - 1-(-phenylcyclopentyl) piperidine
- Schedule I - PCE - N-(1-phenylcyclohexyl) ethylamine (N-ethyl analog of PCP)
- Schedule II – PCP - phencyclidine; 1-(1-phenylcyclohexyl) piperidine
- Schedule II - PCC - 1-piperidinocyclohexane carbonitrile (precursor)

21.3 Extraction

21.3.1 May be extracted from basic or acidic aqueous solution with organic solvents.

21.3.2 May be dry extracted with methanol or other organic solvents.

21.3.3 Plant material samples may be extracted with a suitable solvent (e.g., hexane, methanol) and the extract de -colorized by passing it through a pre-washed Pasteur pipette in which activated charcoal has been placed over a plug of glass wool. The resulting solution may be concentrated and used for further testing. A procedure blank shall be run and documented in the case notes.

21.4 Color Test Results

21.4.1 $Co(SCN)_2$ Results – blue

21.5 TLC

21.5.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

21.5.1.1 TLC1 is recommended for separating the PCP analogs.

21.5.2 Detection methods:

21.5.2.1 Does not show well under UV light due to weak quenching.

21.5.2.2 Detection spray - iodoplatinate

21.6 UV: PCP - triplet with max. at 262 nm in acid

21.7 GC

21.7.1 Analogs can be separated by GC at appropriate temperatures. See GC file for conditions.

21.8 FTIR: Basic extract often results in an oil which may be run as a smear between salt plates.

21.9 PCP Quantitation

21.9.1 See GC ¶ 10 for general quantitation procedure.

21.9.2 Materials

- Chloroform

- Docosane
- PCP or PCP HCl (quantitative standard)
- Class A volumetric flasks
- Analytical balance

21.9.3 Internal standard solution:

21.9.3.1 Prepare a sufficient volume to dilute the PCP standards and all samples.

21.9.3.2 Prepare a 1 mg/mL solution of docosane in chloroform in an appropriate volumetric flask.

21.9.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

21.9.4 PCP standard solutions

21.9.4.1 Weigh at least 10 mg of PCP and quantitatively transfer to a 10 mL volumetric flask with internal standard solution. Fill to mark with internal standard solution. This results in a solution of 1.0 mg/mL PCP in internal standard solution.

21.9.4.2 Prepare a solution of another concentration within the linear range to use as the check standard. Weigh at least 10 mg PCP and quantitatively transfer to volumetric flask. Fill to mark with internal standard solution.

21.9.5 Standard Salt Form Conversion

Unless phencyclidine hydrochloride is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of phencyclidine hydrochloride to free base, multiply the concentration (mg/mL) of PCP HCl by 0.8696 (243.4 F.B./279.9 salt). This will give the concentration of free base in the standard solution.

21.9.6 Sample preparation

21.9.6.1 Prepare two separate sample solutions. For each, weigh approximately 20 mg of sample into a 10 mL volumetric flask. Fill to the mark with internal standard solution.

21.9.7 GC Parameters

- Column: 15 m HP-1 capillary or equivalent (0.25 mm i.d., 0.25 μm film thickness)
- Oven temperature: approximately 210 °C isothermal
- FID temperature: 280 °C

21.9.8 Linear Range

21.9.8.1 The validated linear range of the PCP method is 0.5 – 5.0 mg/mL.

21.9.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

21.9.9 PCP elutes prior to docosane.

21.9.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 10.4.4.7 - 10.4.4.10

22 LYSERGIC ACID DIETHYLAMIDE (LSD) METHODOLOGY

22.1 Scheduling: Schedule I – LSD

22.2 Analog: LAMPA (Lysergic acid methyl propyl amide) is a positional isomer of LSD and is included in Schedule I.

22.3 Extraction

22.3.1 LSD may be dry extracted with Methanol from blotter paper and other matrices.

22.3.2 LSD can be extracted from basic aqueous solution with organic solvents.

22.3.3 It may be necessary to dissolve the samples in a MeOH/$CHCl_3$ mixture to extract LSD out of samples like plastic "window panes."

22.3.4 If samples are in a matrix which is impervious to organic solvents, LSD may be extracted by creating the tartrate salt, followed by base extraction. A procedure blank shall be run and documented in the case notes.

22.3.4.1 Procedure (*Analysis of Drugs* - DEA publication):

- Soak sample in 1% aqueous tartaric acid solution.
- Make basic with sodium bicarbonate powder.
- Extract into chloroform for further analysis.

22.4 Color Test Results

22.4.1 p-DMAB (Erhlich's or Van Urk's) – purple or blue

22.5 TLC

22.5.1 Baths: The TLC8 system is useful to distinguish LSD from LAMPA.

22.5.2 Detection Methods

22.5.2.1 LSD fluoresces blue under long wave UV light.

22.5.2.2 Detection spray - p-DMAB (Ehrlich's or Van Urk's), it may be necessary to heat the plate to get good visualization (Blue spot on a white background).

22.6 UV: Not useful under normal circumstances.

22.7 GC

22.7.1 Very small samples may require reduced split ratio or splitless injection techniques.

22.7.2 LSD and LAMPA separate well on GC.

22.8 FTIR: Not usually possible because of the small quantity of LSD present in samples.

23 MESCALINE METHODOLOGY

23.1 Scheduling

23.1.1 Schedule I – Mescaline (3, 4, 5-Trimethoxyphenylethylamine), which is found in Peyote buttons.

23.1.2 The chemical, rather than the botanical, is controlled.

23.2 Extraction

23.2.1 Dry button and soak in methanol. Filter off plant material prior to analysis.

23.3 Color Test Results

23.3.1 Marquis- orange

23.3.2 Meckes – green → dark brown

23.3.3 Froehdes- green → blue

23.3.4 HNO_3 - bright red

23.4 TLC

23.4.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

23.4.2 Detection spray - iodoplatinate

23.5 UV: Mescaline - max. at 268 nm in acid

24 PSILOCYBIN AND PSILOCYN METHODOLOGY

24.1 Scheduling

24.1.1 Schedule I – psilocybin and psilocyn, which are found in mushrooms.

24.1.2 The chemicals, rather than the botanical, are controlled.

24.2 Extractions

24.2.1 Dry sample (in drying oven or microwave). Grind and soak in methanol for a period of 1 – 24 hours. Filter off mushroom material prior to analysis. This extraction method will allow for the analysis of either psilocyn or psilocybin.

24.2.2 Acetic Acid Extraction Technique (recommended for mushrooms in chocolate or other matrices). This extraction will allow for the analysis of psilocyn. A procedure blank shall be run and documented in the case notes.

- Dry approximately 3 grams material. (Easier to grind when dry.)
- Grind up.
- Let soak in 6% acetic acid for 30 minutes - 1 hour.

- Filter off insoluble material.
- Extract acid portion with three aliquots of $CHCl_3$.* (Discard $CHCl_3$.)
- Basify acid portion with concentrated NH_4OH to pH 8 - 10.
- Extract basic solution with three aliquots of $CHCl_3$.*

- Combine aliquots of $CHCl_3$.
- Evaporate $CHCl_3$ with air (low heat).
- Resultant residue will yield psilocyn.

* Do not mix vigorously in the separatory funnel as an emulsion will probably form.

24.3 Color Test Results

24.3.1 Ehrlich's- purple (positive for psilocyn and psilocybin).

24.3.2 Weber Test- psilocyn - Fast Blue B or Fast Blue BB gives red color; addition of conc. HCl gives blue color.

24.4 TLC

24.4.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

24.4.2 Detection Sprays

24.4.2.1 p-DMAB and HCl - reddish violet area for psilocybin, blue for psilocyn.

24.4.2.2 Weber Test (Fast Blue B or Fast Blue BB) can also be used as a TLC spray to detect psilocyn.

24.5 GC

24.5.1 GC and GC/MS will give only psilocyn due to the dephosphorylation of the psilocybin caused by the GC injection port temperatures.

24.5.2 After methanol extraction and drying, silylation with BSTFA prior to GC or GC/MS will allow differentiation of psilocybin from psilocyn. If psilocybin is to be confirmed, derivatization is required.

24.6 **FTIR:** KBr pellet/ATR on extract from acetic acid extraction yields psilocyn.

25 CATHINONE METHODOLOGY

25.1 Scheduling

25.1.1 Schedule I – cathinone, which is found in *Catha Edulis* (Khat)

25.1.2 Schedule IV – cathine (also known as: (+)-norpseudoephedrine).

25.1.3 The chemical components, rather than the botanical, are controlled.

25.2 Sample Handling

25.2.1 Suspected freshly harvested Khat should be refrigerated upon receipt into the laboratory and subsequently analyzed as soon as possible.

25.2.2 Dried or freeze-dried samples may be refrigerated, but refrigeration is not required.

25.2.3 Cathinone can enzymatically convert to Cathine.

25.3 Extraction

A procedure blank shall be run with either extraction and documented in the case notes.

25.3.1 Suggested Khat extraction technique for freshly harvested plant material

- Cut up the leaves and stems to obtain the sample. The sample size should be based upon the amount submitted, and the need to retain material for reanalysis if requested.
- Homogenize or sonicate the material for 5 minutes in enough 0.1N HCl to cover the sample
- Filter off insoluble material
- Basify the solution with 1N NaOH to pH 11-12
- Extract basic solution with aliquots of $CHCl_3$.
- Evaporate combined $CHCl_3$ aliquots with air to dryness
- Reconstitute sample with an appropriate solvent
- Analyze residue as soon as possible or refrigerate to avoid degradation.
- Resulting sample should contain Cathinone and Cathine.

25.3.2 Suggested Khat extraction technique for dried plant material

- The sample size should be based upon the amount submitted, and the need to retain material for reanalysis if requested. Dried material need not be cut up.
- Sonicate or soak dried material in enough 0.1N HCl to cover the sample, for 5 minutes
- Filter off insoluble material
- Basify the solution with 1N NaOH to pH 11-12
- Extract basic solution with aliquots of $CHCl_3$.
- Evaporate combined $CHCl_3$ aliquots with air to dryness
- Reconstitute sample with an appropriate solvent
- Analyze residue as soon as possible or refrigerate to avoid degradation.
- Resulting sample should contain Cathinone and Cathine.

25.4 TLC

25.4.1 Baths: TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.

25.4.2 Cathinone and Cathine reference standards shall be run on the plate for comparison.

25.4.3 Detection Spray: Ninhydrin/heat gives a red-brown color and shall be used for visualization.

25.5 GC/MS: Consult standard verification files for conditions.

25.5.1 It is recommended that the Cathine standard be run along with the Cathinone standard to illustrate spectral differences.

25.5.2 Derivatization may also be useful in increasing retention time difference between Cathinone and Cathine, as well as providing additional spectral information. Acetylation and Silylation are recommended.

25.6 References

25.6.1 Morselli *et al.*, "Gas-Chromatography/Mass Spectrometry Determination of the Active Principles of (Catha Edulis) African Vegetable," *Microgram*, Vol. XXV, No. 11, November 1992, pp. 290-294.

25.6.2 Lee, M. M., "The Identification of Cathinone in Khat (Catha edulis): A Time Study," *Journal of Forensic Sciences*, JFSCA, Vol. 40, No. 1, January 1995, pp. 116-121.

25.6.3 Chappell, John S., Lee, Marsha M., "Cathinone preservation in khat evidence via drying," *Forensic Science International,* 195 (2010), pp. 108-120.

25.6.4 LeBelle et al., "Gas chromatographic-mass spectrometric identification of chiral derivatives of the alkaloids of Khat," *Forensic Science International*, 61 (1993), pp. 53-64.

25.6.5 Lehmann et al., "Rapid TLC Identification Test for Khat (*Catha Edulis*)," *Forensic Science International*, 45 (1990), pp. 47-51.

25.6.6 Ripani et al., "GC/MS identification of Catha edulis stimulant-active principles," *Forensic Science International*, 78 (1996), pp. 39-46.

26 MDA/MDMA METHODOLOGY

26.1 Scheduling

- Schedule I - 3,4-methylenedioxyamphetamine (MDA)
- Schedule I - 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy)
- Schedule I - 3,4-methylenedioxy-N-ethylamphetamine (MDEA, Eve)
- Schedule I - 4-bromo-2,5-dimethoxyphenethylamine (2C-B, Nexus)

26.2 Color Test Results

26.2.1 The sulfuric acid series of color tests generally give intense colors that undergo vivid transitions with MDA and MDMA. These may all appear black with very concentrated samples.

26.2.2 Marquis

- MDA/MDMA - dark violet → black
- Nexus – light green → green

26.2.3 Meckes

- MDA/MDMA - green → dark blue/violet → black
- Nexus - yellow

26.2.4 Frochdes

- MDA/MDMA - brown → dark blue/violet → black
- Nexus – yellow

26.2.5 TBPEE

- MDA – purple
- MDMA – blue
- MDEA - blue
- Nexus – purple

26.3 TLC

26.3.1 Baths:

- TLC1, TLC2, TLC3, TLC4 and TLC5 are recommended.
- TLC9 separates methamphetamine from MDMA.

26.3.2 Detection sprays

- Iodoplatinate, results may be enhanced by overspraying with Ceric Sulfate.
- Dragendorff
- Fluram visualizes MDA, Nexus and other primary amines.

26.4 UV: MDA/MDMA - maximum at 234 nm and 285 nm in acid with associated minima.

26.5 GC

26.5.1 Extraction of the sample may be necessary to get good chromatography.

26.5.2 Acetyl Derivative: The acetyl derivative of MDMA-type compounds is made by drawing up 1 µL of sample followed by 1 µL of acetic anhydride, separated by an air bubble. The acetyl derivative should have a longer retention time than the underivatized compound and may require a higher temperature than the underivatized compound.

26.6 FTIR

26.6.1 Extraction from excipients may be necessary to obtain a good spectrum.

26.6.2 GC-FTIR is a useful tool to differentiate MDMA-type compounds.

26.7 MDMA Quantitation

26.7.1 See GC ¶ 10 for general quantitation procedure.

26.7.2 Materials:

- Methylene Chloride or Chloroform
- Octadecane
- 3,4-MDMA HCl
- 4N NaOH
- Class A volumetric pipettes
- Class A volumetric flasks
- Analytical balance

26.7.3 Internal Standard Solution:

26.7.3.1 Prepare a sufficient volume to dilute the standard solutions and all samples.

26.7.3.2 Prepare a 1 mg/mL solution of octadecane in methylene chloride or chloroform in the appropriate volumetric flask.

26.7.3.3 Refrigerated solutions should be allowed to return to ambient temperature prior to use.

26.7.4 MDMA Standard Solutions:

26.7.4.1 Prepare a 2 mg/mL standard

- Weigh approximately 20 mg of the standard
- Dilute to approximately 5 mL with D.I. or R.O. water
- Make basic with 4N sodium hydroxide until pH is above 10
- Use a volumetric pipette to deliver the internal standard solution
- Extract two times with 5 mL portions of internal standard solution
- Combine the extracts for further analysis

26.7.4.2 Prepare a solution of another concentration within the linear range to use as the check standard. Weigh at least 10 mg of standard and combine the extracts from two extractions as outlined above.

26.7.5 Standard Salt Form Conversion

Unless MDMA HCl is to be reported with its salt form, the concentration in its base form will be calculated. To convert the concentration of MDMA HCl to free base, multiply the concentration (mg/mL) of MDMA HCl by 0.8413 (193.25 F.B./229.71 HCl). This will give the concentration of free base in the standard solution.

26.7.6 Sample Preparation:

Prepare and extract two separate sample solutions for a duplicate analysis. For each, weigh approximately 20 -40 mg of the sample, dilute to 5 mL with water and extract as with the standard solution. Run a duplicate analysis by preparing two separate solutions.

26.7.7 GC parameters:

- Column: 15 m HP-1 capillary (0.25 mm i.d. 0.25 μm film thickness).
- Oven temperature: approximately 170 - 240°C
- FID temperature : 280°C

26.7.8 Linear Range

26.7.8.1 The validated linear range of the MDMA method is 0.5 – 5 mg/mL.

26.7.8.2 Once the percentage purity has been calculated for the sample, verify that the calculated concentration of the sample was within the linear range of the method. If it was outside the linear range, remake the sample solutions using a more appropriate amount of material.

26.7.9 Octadecane elutes after MDMA.

26.7.10 Calculations, Acceptance Criteria and Reporting

See ¶¶ 10.4.4.7 - 10.4.4.10

27 ANABOLIC STEROID METHODOLOGY

27.1 Brief Pharmacology: Promotes muscle growth (anabolic effect)

27.2 Drug Group Examples: Testosterone (associated esters), stanozolol, boldenone

27.3 Scheduling: Anabolic steroids are listed in Schedule III.

27.4 Extraction

27.4.1 May be dry extracted into methanol or other organic solvents.

27.4.2 Injectables are often found in oils which may be extracted with methanol for further analysis.

27.5 Color Test Results: There are no good screening tests for steroids.

27.6 Pharmaceutical Identifiers

27.6.1 Many substituted or negative preparations are encountered which make pharmaceutical identifiers less useful than with other types of preparations. They should not be ignored, but may need to be discounted.

27.7 TLC

27.7.1 Baths: TLC6 separates many anabolic steroids. TLC1, TLC2, TLC3, TLC4 and TLC5 are also recommended.

27.7.2 Detection methods:

- UV
- Sulfuric Acid/Ethanol Reagent for steroids
- Iodoplatinate will visualize Stanozolol
- $KMnO_4$ will visualize steroids with unsaturated bonds

27.8 GC

27.8.1 Some of these materials will require elevated temperatures and have long retention times. Special derivatizing techniques may assist chromatographic performance.

27.9 FTIR: May need additional extraction to eliminate oils.

27.10 GC/MS

27.10.1 Molecular weights may exceed 500 and the usual mass spectral mass range would then need to be extended.

27.11 References

27.11.1 Chiong *et al.* "The Analysis and Identification of Steroids", *Journal of Forensic Sciences*, March 1992.

27.11.2 Walters *et al.* "Analysis of Illegally Distributed Anabolic Steroid Products", *JAOAC*, Vol. 73, No. 6, 1990.

28 GHB METHODOLOGY

28.1 Brief Pharmacology: Central nervous system depressant

28.2 Drug Group Examples

28.2.1 Gamma hydroxybutyric acid (GHB), gamma-butyrolactone (GBL) and 1,4-butanediol (BD)

28.2.2 Other names for gamma hydroxybutyric acid include gamma hydroxybutyrate; 4-hydroxybutyrate; 4-hydroxybutanoic acid; sodium oxybate; and sodium oxybutyrate.

28.3 Scheduling

- Schedule I : GHB (not found in an approved drug product)
- Schedule III : Any drug product containing gamma hydroxybutyric acid, including its salts, isomers, and salts of isomers, for which an application is approved under section 505 of the Federal Food, Drug, and Cosmetic Act
- Enhanced penalty: Possession and distribution of gamma-butyrolactone and 1,4-butanediol when intended for human consumption is a Class 3 felony.

28.4 Chemical Properties

28.4.1 GHB: Pure GHB is a white powder. It is encountered dissolved into various liquids.

28.4.2 GBL: Pure GBL is a clear liquid. It is encountered dissolved into various liquids.

28.4.3 BD: Pure BD is a viscous clear liquid.

28.5 pH

28.5.1 If the sample is in a liquid form, take the pH of the solution prior to beginning analysis. GHB is generally found in basic solutions while GBL is generally found in acidic solutions. However, equilibrium occurs between the two in solution.

28.6 Color Test Results

28.6.1 Ferric Chloride – GHB will turn red-brown (Results can vary depending on sample pH and liquid matrix. Therefore, further screening is necessary.)

28.6.2 GHB Color Test #3 (Smith Test) – GHB – immediate green

28.7 TLC

28.7.1 Bath: TLC3 and TLC10 (Ethyl Acetate) are recommended.

28.7.2 Detection: Iodine Vapors Results (in TLC10)

- GHB off-white spot at origin
- GBL brown spot near solvent front
- BD off-white to brown bearding spot midway up plate

28.8 GC

28.8.1 GHB will form gamma-butyrolactone (GBL) in the heated injection port. The silyl derivative, prepared prior to injection, is required to differentiate GHB from GBL.

28.8.1.1 If the solution contains a mixture of GBL and GHB, perform multiple chloroform rinses of the solution to remove the GBL prior to derivatization. GHB is not soluble in chloroform.

28.8.1.1.1 This chloroform extract containing GBL can be used for GC retention time comparison with a GBL standard.

28.8.1.2 Monitor the chloroform extracts by GC/MS to see when the majority of the GBL has been removed. After performing the extracts, dry down your sample under an air stream and/or in a vacuum oven without heat. Then, derivatize your sample with BSTFA with 1% TMCS.

28.8.2 BD does not chromatograph well and may breakdown (lose water) in the heated injection port.

28.9 GC/MS

28.9.1 GHB and BD - Derivatize dry sample with BSTFA with 1% TMCS. See MS file for conditions.

28.9.2 GBL: A chloroform extract of a liquid containing GBL can be used to confirm GBL by GC/MS.

28.10 FTIR

28.10.1 GHB: Direct KBr pellet or ATR on powder sample for GHB.

28.10.2 BD: Light liquid smear on prepared KBr pellet or ATR.

28.10.3 GBL: Light liquid smear on prepared KBr pellet or ATR. If results are unfavorable, perform a chloroform extract of liquid. Use this extract to prepare either a liquid smear on a prepared KBr pellet or dry it on KBr prior to making a pellet. Alternatively, the ATR may be used.

28.10.4 GHB/GBL Mixtures:

For liquid mixture samples of GHB and GBL, it may be necessary to separate the GHB from the matrix and/or accompanying GBL using preparatory TLC. Streak the origin of a TLC plate with the liquid sample, run in TLC10, vacuum/scrape off silica gel at origin, and elute GHB from silica gel with multiple rinses of the same methanol extract. Dry down the methanol extract and prepare a KBr pellet of the resultant powder.

28.11 References

28.11.1 Ciolino, L. A. et al. "The Chemical Interconversion of GHB and GBL" Forensic Issues and Implications" *Journal of Forensic Sciences*, 2001, Vol. 46, No. 6, pp. 1315-1323.

28.11.2 Bommarito, C. "Analytical Profile of Gamma-Hydroxybutyric Acid (GHB)" *Journal of the Clandestine Laboratory Investigating Chemists Association*, Vol. 3, No. 3, 1993.

28.11.3 Chappell, J. S. "The Non-equilibrium Aqueous Solution Chemistry of Gamma-Hydroxybutyric Acid" Journal *of the Clandestine Laboratory Investigating Chemists Association*, Vol. 12, No. 4, 2002.

29 SALVINORIN A METHODOLOGY

29.1 Scheduling

29.1.1 Schedule I – Salvinorin A, which is found in *Salvia divinorum*

29.1.2 The chemical, rather than the botanical, is controlled.

29.2 Extractions

29.2.1 Dry sample (in drying oven or microwave) if not already in a dried form.

29.2.2 Soak approximately 50-100 mg of plant material in CH_2Cl_2 for at least 30 minutes. CH_2Cl_2 is the recommended solvent as it is the most efficient extraction solvent of Salvinorin A and the least efficient extraction solvent of other plant component interferences. Chloroform and methanol may also be used. Hexane does not effectively extract Salvinorin A and basic extractions may hydrolyze the ester groups on other salvinorins present and should be avoided. A procedure blank shall be run for multi-step extractions and documented in the case notes.

29.2.3 For smaller samples or suspected weak samples, sonicating the plant material in the solvent may help increase the efficiency of the extraction.

29.2.4 Filter off plant material prior to analysis.

29.2.5 Concentrate the extraction solvent into an autosampler vial for analysis.

29.2.6 Residues

29.2.6.1 Rinse suspected residues with CH_2Cl_2 and concentrate into an autosampler vial insert.

29.2.6.2 A reduced split of 20:1 on GC and GC/MS may be necessary to concentrate the sample sufficiently for identification. Vials should be returned with the evidence as outlined in ¶ 5.7.2.

29.3 TLC

29.3.1 Baths: TLC14 (see ¶ 9.4) separates Salvinorin A from Salvinorin B/D and C (see reference 29.6.4). Use TLC1 or TLC2 for the additional bath.

29.3.2 Detection Sprays:

29.3.2.1 Vanillin spray (see ¶ 9.5.16)

- For plate developed in the basic TLC2 bath, spray plate with 6N HCl prior to Vanillin.
- Generously spray Vanillin on developed and dried plate
- Heat with heat gun or in oven to 110 °C for several minutes
- Pinkish purple spots will develop for salvinorins

29.4 GC and GC/MS

29.4.1 GC and GC/MS

- Split 50:1 or less
- HP-5 or HP-1 or equivalent
- Approximate temperature range - 240-300 degrees C at 30/min
- MS scan range - 500-14 Da.

29.4.2 Salvinorin A is the most abundant salvinorin in *Salvia divinorum*. The other salvinorins are much less concentrated and will elute on either side of Salvinorin A.

29.5 Reporting

See ¶ 33.

29.6 References

29.6.1 Giroud C, Felber F, Augsburger M, Horisberger B, Rivier L, Mangin P. "*Salvia divinorum*: an hallucinogenic mint which might become a new recreational drug in Switzerland". Forensic Science International 112(2000) 143-150.

29.6.2 Jermain JD. "Analyzing *Salvia divinorum* and its active ingredient Salvinorin A utilizing thin layer chromatography and gas chromatography/mass spectrometry". Permission from author March 2008, CLIC list.

29.6.3 Medana C, Massolino C, Pazzi M, Baiocchi C. "Determination of salvinorins and divinatorins in *Salvia divinorum* by liquid chromatography/multistage mass spectrometry". Rapid Commun. Mass Spectrum 2006; 20:131-136.

29.6.4 Siebert DJ. "Localization of Salvinorin A and Related Compounds in Glandular Trichomes of the Psychoactive Sage, *Salvia divinorum*". *Annuals of Botany* 2004;93:763-771.

29.6.5 Wolowich WR, Perkins AM, and Cienki JD. "Analysis of the Psychoactive Terpenoid Salvinorin A content in five *Salvia divinorum* herbal products". *Pharmacotherapy* 2006;26(9): 1268-1272.

30 SYNTHETIC CANNABINOID METHODOLOGY

30.1 Scheduling

30.1.1 Code of Virginia, §18.2-248.1:1 defines synthetic cannabinoids as "cannabimimetic agents" which are compounds that are either listed specifically or fall within one of the defined structural classes.

30.1.1.1 Structural classes

- 2-(3-hydroxycyclohexyl)phenol with substitution at the 5-position of the phenolic ring by alkyl or alkenyl, whether or not substituted on the cyclohexyl ring to any extent
- 3-(1-naphthoyl)indole or 1H-indol-3-yl-(1-napthyl)methane with substitution at the nitrogen atom of the indole ring, whether or not further substituted on the indole ring to any extent, whether or not substituted on the naphthoyl or naphthyl ring to any extent
- 3-(1-naphthoyl)pyrrole with substitution at the nitrogen atom of the pyrrole ring, whether or not further substituted in the pyrrole ring to any extent, whether or not substituted on the naphthoyl ring to any extent
- 1-(1-naphthylmethyl)indene with substitution of the 3-position of the indene ring, whether or not further substituted in the indene ring to any extent, whether or not substituted on the naphthyl ring to any extent
- 3-phenylacetylindole or 3-benzoylindole with substitution at the nitrogen atom of the indole ring, whether or not further substituted in the indole ring to any extent, whether or not substituted on the phenyl ring to any extent

30.1.1.2 The following list contains the synthetic cannabinoids specifically listed under Code of Virginia, §18.2-248.1:1. There are many potential isomers of the listed compounds.

- 5-(1,1-Dimethylheptyl)-2-[3-hydroxycyclohexyl]-phenol (other name: CP 47,497)
- 5-(1,1-Dimethylhexyl)-2-[3-hydroxycyclohexyl]-phenol (other name: CP 47,497 C6 homolog)
- 5-(1,1-Dimethyloctyl)-2-[3-hydroxycyclohexyl]-phenol (other name: CP 47,497 C8 homolog)
- 5-(1,1-Dimethylnonyl)-2-[3-hydroxycyclohexyl]-phenol (other name: CP 47,497 C9 homolog)
- 1-pentyl-3-(1-naphthoyl)indole (other name: JWH-018)
- 1-butyl-3-(1-naphthoyl)indole (other name: JWH-073)
- 1-pentyl-3-(2-methoxyphenylacetyl)indole (other name: JWH-250)
- 1-hexyl-3-(naphthalen-1-oyl)indole (other name: JWH-019)
- 1-[2-(4-morpholinyl)ethyl]-3-(1-naphthoyl)indole (other name: JWH-200)
- (6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol (other name: HU-210)
- 1-pentyl-3-(4-methoxy-1-naphthoyl)indole (JWH-081);
- 1-pentyl-3-(4-methyl-1-naphthoyl)indole (JWH-122);
- 1-pentyl-3-(2-chlorophenylacetyl)indole (JWH-203);
- 1-pentyl-3-(4-ethyl-1-naphthoyl)indole (JWH-210);
- 1-pentyl-3-(4-chloro-1-naphthoyl)indole (JWH-398);
- 1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole (AM-694);
- 1-((N-methylpiperidin-2-yl)methyl)-3-(1-naphthoyl)indole (AM-1220);
- 1-(5-fluoropentyl)-3-(1-naphthoyl)indole (AM-2201);
- 1-[(N-methylpiperidin-2-yl)methyl]-3-(2-iodobenzoyl)indole (AM-2233)
- Pravadoline (4-methoxyphenyl)-[2-methyl-1-(2-(4-morpholinyl)ethyl)indol-3-y]methanone

(WIN 48,098);
- 1-pentyl-3-(4-methoxybenzoyl)indole (RCS-4);
- 1-(2-cyclohexylethyl)-3-(2-methoxyphenylacetyl)indole (RCS-8)

30.1.2 The compounds themselves, rather than the herbal blends in which they are commonly found, are controlled.

30.1.3 Under §18.2-248.1:1(F), analogs of the listed synthetic cannabinoids are also subject to the same criminal penalties.

30.2 Extractions

30.2.1 Extract sample into a suitable solvent (e.g., methanol, $CHCl_3$ or hexane).

30.3 TLC

30.3.1 Baths: TLC1, TLC2, or TLC5 are recommended.

30.3.2 Detection Sprays:

- $KMnO_4$ (Ceric Sulfate or 6 N HCl may be used as an overspray)
- Fast Blue B or BB overspray with 6 N HCl

30.3.3 Due to the limitations of TLC in distinguishing isomers, two system GC must be run to determine if more than one isomer is present in the sample.

30.4 GC and GC/MS

30.4.1 Two system GC must be utilized.

30.4.2 GC and GC/MS

- Split 60:1
- Columns: HP-5, HP-35 ((35% phenyl)-methylpolysiloxane) and HP-1 or equivalent
- Approximate temperature range 225-300 degrees C at 30/min, although broader temperature ranges may be indicated
- MS scan range, 500-14 Da or 600-14 Da

30.5 References

30.5.1 Rainer Lindigkeit, Anja Boehme, Ina Eiserloh, Maike Luebbecke, Marion Wiggermann, Ludger Ernst, Till Beuerle, "Spice: A Never Ending Story?", *Forensic Science International*, 191 (2009), pp. 58-63.

30.5.2 *Journal of Mass Spectrometry* (2009), JMS Letter via www.interscience.wiley.com/journal/jms.

30.5.3 National Forensic Laboratory Information System (NFLIS), Drug Enforcement Administration (DEA), *Year 2008 Annal Report*, DEA Update "Spice – Request for Information", pg. 5.

30.5.4 "Identification of Synthetic Cannabinoids in Herbal Incense Blends by GC/MS" Application Compendium, Agilent Technologies, 5990-7967EN, 2011.

30.5.5 Nahoko Uchiyama, Maiko Kawamura, Ruri Kikura-Hanajiri, Yukihiro Goda, "Identification and quantitation of two cannabimimetic phenylacetylindoles JWH-251 and JWH-250, and four cannabimimetic naphthoylindoles JWH-081, JWH-015, JWH-200, and JWH-073 as designer drugs in illegal products", *Forensic Toxicology*, DOI 10.1007/s11419-010-0100-3 (Nov 2010).

30.5.6 Malinda Combs and Jeremiah A. Morris, "Analytical Profile of Two Synthetic Cannabinoids – JWH–018 and JWH–073", *Journal of the Clandestine Laboratory Investigating Chemists Association*, Vol. 20(2) (April 2010), pp. 2-7.

30.5.7 N. Uchiyama, et al., "Chemical analysis of synthetic cannabinoids as designer drugs in herbal products", Forensic Sci. Int. (2010), doi:10.1016/j.forsciint.2010.01.004

30.5.8 "Journal of Mass Spectrometry Letter "'Spice' and other herbal blends: harmless incense or cannabinoid designer drugs?" (www.interscience.com) DOI 10.1002/jms.1558, (2009).

30.5.9 Code of Virginia, §18.2-248.1:1

31 CLANDESTINE LABORATORIES

31.1 Introduction

31.1.1 Analysis of samples collected from clandestine labs may involve the use of both routine and non-routine analytical techniques. Analysis and subsequent identification of inorganic compounds, including acids and bases, may require the transfer of certain items to Trace Evidence. For examiners qualified in both the Controlled Substances and Trace Evidence General Chemical disciplines, the results from both should be reported on one Certificate of Analysis.

31.1.2 Due to the nature of clandestine laboratories, it is not uncommon for a relatively large number of items to be submitted. In order to determine the best analytical approach, it may be necessary to confer with a supervisor, the investigating officer, and the prosecuting attorney to assess the probative value of each piece of submitted evidence. Refer to the DFS "Evidence Handling and Laboratory Capabilities Guide" under the "Controlled Substances – Clandestine Laboratories" section for guidelines on evidence collection, evidence packaging, and evidence submission.

31.1.3 Occasionally, clandestine labs may be processed by a federal agency or in conjunction with a federal agency. Evidence collected by federal agencies for clandestine lab cases that will be prosecuted in a Federal venue should be submitted to a federal laboratory for analysis.

31.1.4 The evidence submitted for a clandestine laboratory investigation can pose significant health hazards that are not commonly encountered with routine controlled substance examinations. These hazards may include but are not limited to: corrosives, caustic materials, explosives, toxic gases, and flammable solvents. Due caution should be exercised when opening and examining evidence of this nature by utilizing appropriate personal protective equipment and sampling in appropriately ventilated areas (e.g., fume hood). Every effort should be made to prevent exposure of other employees to potentially hazardous materials. Special storage precautions may be necessary.

31.2 Analytical Approach

31.2.1 Ideally, the submitted items of evidence should collectively contain the necessary components to fully demonstrate either the intent to manufacture or the successful manufacture of a controlled substance. In addition to the controlled substance which is suspected to be the target product, precursors and essential chemicals should be identified when present.

31.2.1.1 When the suspected target product is methamphetamine, methcathinone, or amphetamine, it is important to attempt to identify at least two of the substances listed in §18.2-248(J). The identification of many of the chemicals on this list will require analysis by the Trace Evidence section.

31.2.2 The evidence that has been determined to have the greatest probative value should be sampled and analyzed first. The Drug Item Reduction Policy should be followed once sufficient analysis has been performed to support the charge on the RFLE.

31.2.3 If possible, the investigating officer should be encouraged to provide a copy of any notes or procedures found at the clandestine laboratory scene to aide in the identification of synthetic routes.

31.3 Procedure

31.3.1 Solid Materials and Powders

31.3.1.1 Solid materials and powders should be sampled and analyzed following the schematic illustrated in ¶ 2.2 of the Procedures Manual. Samples submitted in the course of clandestine laboratory investigations may require additional analysis.

31.3.1.2 If the sample is soluble in water, cation and anion analysis may be performed for screening purposes by using the assays for specific ions found in the U.S. Pharmacopeia.

31.3.1.3 If a solid sample or powder is soluble in water, record the pH of an aqueous solution made from a portion of the sample.

31.3.1.4 Solid materials and powders that are most likely an inorganic essential chemical (e.g., iodine, red phosphorus) should be transferred to the Trace Evidence section for identification.

31.3.2 Liquid Samples

31.3.2.1 Liquids should be sampled and analyzed following the procedure in ¶ 2.11 of the Procedures Manual. Samples submitted in the course of clandestine laboratory investigations may require additional analysis.

31.3.2.2 Presumptive identification of suspected ionized species in aqueous solution may be achieved by using the assays for specific cations and anions found in the U.S. Pharmacopeia.

31.3.2.3 The miscibility of liquid samples with both water and a water insoluble solvent (e.g., $CHCl_3$, Hexane) should be determined.

31.3.2.4 For liquids with multiple layers, care should be taken to note the number of layers, the location of each layer relative to the others, the color and clarity of each layer. When liquid samples with multiple layers are encountered each layer shall be sampled and analyzed.

31.3.2.5 When a precipitate is discovered in a liquid sample, the precipitate should be sampled and analyzed in addition to or in conjunction with the liquid.

31.3.2.6 Liquid samples that are most likely an essential chemical (e.g., a brown liquid in a labeled "Tincture of Iodine" bottle) should be treated as a Trace Evidence General Chemical examination or transferred to the Trace Evidence section for identification.

31.3.3 A definitive instrumental analysis technique must be employed to identify a drug, precursor or essential chemical. The relevant section of the Procedures Manual for the suspected target drug may help to clarify potential analytical issues that arise during the course of the clandestine laboratory analysis. Significant deviations from routine analytical procedures must be documented in accordance with QM ¶ 5.3.10. Non-routine analytical procedures shall be clearly documented in the examination documentation along with proper approval from the Section Supervisor and the Chemistry Program Manager, if appropriate.

31.4 References

31.4.1 "Clandestine Lab Basic Guide" presented 12th Annual Training Seminar, Clandestine Laboratories Investigating Chemists, New Orleans, LA Sept. 4-7, 2002

31.4.2 U.S. Pharmacopeia National Formulary

32 ESTIMATION OF THE UNCERTAINTY OF MEASUREMENT (UOM)

32.1 Policy

32.1.1 The Department will develop processes and collect data for the implementation of the below listed policy (¶ 32 in its entirety). The Department will conform to the timely implementation of this policy congruent with current accreditation guidelines.

32.1.2 An estimation of UOM shall be determined for sample weights that are reported on the Certificate of Analysis.

32.1.3 An estimation of UOM shall be determined for critical quantitation results that are reported on the Certificate of Analysis. A quantitation result is considered critical when it is being reported to fulfill a statutory requirement or sentencing guideline. Current analyses which fall into this category involve methamphetamine, amphetamine, phencyclidine (PCP) and tetrahydrocannabinol in hash oil.

32.2 Estimating the Uncertainty of Measurement

32.2.1 Uncertainty Budget

32.2.1.1 Estimations of the uncertainty of measurement shall be conducted and documented using an uncertainty budget *(Uncertainty Budget Form)*.

32.2.1.2 The uncertainty budget for a given procedure shall include both random (Type A) uncertainties and systematic (Type B) uncertainties.

32.2.1.3 Since the uncertainty of measurement is only an estimate, generally uncertainties shall not exceed two significant figures.

32.2.1.4 To be conservative, calculations used to estimate the uncertainty and the final combined uncertainty should be rounded up.

32.2.1.5 In order to combine the uncertainty, the units of uncertainty values should be measured in the same units.

32.2.1.6 Uncertainty budgets should be re-evaluated on an annual basis.

32.2.2 Random (Type A) Uncertainties

32.2.2.1 Random (Type A) uncertainty results from measurement values being scattered in a random fashion due to laws of chance, thus has a normal or Gaussian shaped distribution.

32.2.2.2 Random (Type A) uncertainty is best determined by historical data of a large number of repeated measurements.

32.2.3 Systematic (Type B) Uncertainties

32.2.3.1 Systematic uncertainty results from the inherent biases in measuring systems and quantitative analytical methods. These uncertainties may be reduced by optimizing the method or measuring system, but can never be completely eliminated.

32.2.3.2 Systematic (Type B) uncertainties resulting from measurement bias typically have an equal chance of falling within a range and therefore follow a rectangular or random distribution.

32.2.3.2.1 With rectangular distribution, the range (±a) of the outer limits is used to estimate the standard deviation (σ) using the equation $\sigma = a/\sqrt{3}$.

32.2.3.2.2 For example, a 10 mL volumetric flask has a tolerance of ±0.2 mL. The calculated uncertainty associated with this measurement is $0.2/\sqrt{3}$ or 0.115. To maintain only 2 significant figures, the uncertainty for this measurement used in the uncertainty budget is 0.12 (after rounding up).

32.2.4 Determination of Combined Uncertainty

32.2.4.1 Uncertainties are combined using the Root Sum Squares technique

$$U_{combined} = \sqrt{(U_1^2 + U_2^2 + U_3^2 + U_4^2 ....)}$$

32.2.5 Determination of expanded uncertainty and confidence

32.2.5.1 In order to determine the expanded uncertainty, the combined uncertainty is multiplied by the coverage factor (k) using the equation $U_{expanded} = U_{combined} \times k$

32.2.5.2 For routine measurements with a large amount of historical data ($n \geq 100$)

32.2.5.2.1 The coverage factor for 95% confidence is k = 2

32.2.5.2.2 The coverage factor for 99.8% confidence is k = 3

32.2.5.3 For analysis with reduced confidence due to lack of historical data, a corrected coverage factor (k_{corr}) is used based on the Student's t table.

32.2.5.3.1 For example, for an analysis with no historical control data, a control is analyzed 15 times (degrees of freedom or df = n-1, or 14 in this example).

32.2.5.3.2 Using the Student's t table, k_{corr} value of 3.79 would be used to calculate the expanded uncertainty at 3σ or 99.8% confidence limit.

32.3 Weights

32.3.1 An uncertainty budget shall be completed which will include both random (Type A) uncertainty and systematic (Type B) uncertainty for each balance.

32.3.1.1 Random uncertainty will be the standard deviation (σ) of 100 measurements of one weight generated from the weekly QA data.

Balance Type	Weight
Analytical (4 place)	1 g
Top Loading (3 place)	100 g
Top Loading (2 place)	100 g
High Capacity (g)	1000 g
High Capacity (kg)	10 kg

32.3.1.2 Systematic uncertainty will include consideration of digital balance resolution, corner loading (shift test) and uncertainty of the calibration check weight. Systematic uncertainties given without a confidence level should be treated as rectangular distributions, and as such will be divided by the $\sqrt{3}$ prior to combining. (see 36.32)

32.3.2 The combined uncertainty for the balance will be calculated using the Root Sum Squares method.

32.3.3 Calculate the expanded uncertainty with a confidence level of 99.8% by using a value of k=3. This final uncertainty value will be used for the calculating the uncertainty of weights in casework.

32.3.4 Uncertainty Budget Example for Weighing with Top Loading Balance (2 place)

Source of Uncertainty	Value (units)	Distribution	Divisor	Uncertainty	K corr
Type A					
Balance Repeatability (σ of 100 measurements)	0.0062 g	Normal	1	0.01 (rounded up from 0.0062 due to sig figs)	3
Type B					
Balance Resolution (digital)	0.01 g	Rectangular	√3	.01 (rounded up from 0.006 due to sig figs)	
Shift test (corner loading)	0.01 g	Rectangular	√3	.01 (rounded up from 0.006 due to sig figs)	
Calibration Check Weight	0.001 g	Rectangular	√3	negligible	
$U_{combined} = \sqrt{(U_1^2 + U_2^2 + U_3^2)}$				0.02 (rounded up from 0.017)	
$U_{expanded} = U_{combined} * 3$ k=3				± 0.06 g	
Confidence = 99.8%					

32.3.5 Items with Single Specimens

32.3.5.1 The calculated expanded uncertainty is the uncertainty for that measurement.

32.3.6 Items with Multiple Specimens

32.3.6.1 When weights are added to calculate a total weight, the uncertainties associated with each individual value must be taken into account in the total uncertainty. The Root Sum Squares method is used for this calculation.

32.3.6.2 Example: The contents of five plastic bag corners are weighed and the individual weights are added together. The estimated expanded uncertainty of measurement for the top-loading balance used is ± 0.06 grams (see budget example above).

1.25 ± 0.06 grams
1.95 ± 0.06 grams
2.20 ± 0.06 grams
1.35 ± 0.06 grams
+ 1.50 ± 0.06 grams
8.25 ± total expanded uncertainty

Total Expanded Uncertainty = $\sqrt{(0.06^2 * 5)} = 0.14$ grams

Total weight = 8.25 ± 0.14 grams

Reported weight = 8.11 grams

32.4 Case File Records and Reporting for Weights

32.4.1 The estimated uncertainty will be subtracted from the recorded weight prior to reporting.

32.4.1.1 Calculations to arrive at the reported weight shall be recorded in the case notes.

32.4.1.2 For total weights, the total expanded uncertainty will be subtracted from the recorded total weight.

32.4.1.3 The last decimal place will be truncated prior to reporting.

32.4.1.4 The *UOM Weight Worksheet* will be used to record calculations determining the reported weight and will be stored in the case file.

32.5 Quantitations

32.5.1 An uncertainty budget shall be completed which will include both random (Type A) uncertainty and systematic (Type B) uncertainty for methods measuring amphetamine, methamphetamine, PCP and tetrahydrocannabinol in hash oil.

32.5.2 Random (Type A) uncertainty values will include control data collected from each laboratory.

32.5.3 Systematic uncertainty will include consideration of glassware uncertainty, standard purity uncertainty, and balance uncertainty. Systematic uncertainties given without a confidence level should be treated as rectangular distributions, and as such will be divided by the √3 prior to combining. (see 36.32)

32.5.4 The combined uncertainty will be calculated using the Root Sum Squares method.

32.5.5 Calculate the expanded uncertainty with a confidence level of 99.8% by using a value appropriate to the number of measurements (see 32.2.5.3).

32.5.6 Calculations to arrive at the reported quantitation result shall be recorded in the case notes.

32.5.7 The estimated uncertainty will be subtracted from the quantitation result prior to reporting.

33 REPORTING GUIDELINES

33.1 Controlled substances are to be reported simply with wording consistent with the Code of Virginia.

33.1.1 The identity and schedule will be reported whenever clearly known.

33.1.2 The "show form" option will be utilized for most types of evidence including plant material, powders and solid material.

33.1.3 Items consisting of one tablet or capsule which were chemically identified will be reported as outlined in ¶ 33.4.1.

33.1.4 Results shall be specific to the item tested. Additional language should be added to the results to clarify which specimen was tested when more than one specimen is present in the item (e.g., the item consists of two pipes so the results clarify that only one was tested.)

33.1.5 The condition of the outer packaging shall be on the Certificate of Analysis.

33.2 When residue samples contain controlled substances or marijuana, the results section must reflect the term "residue."

33.2.1 Examples:

- Marijuana, residue
- Heroin, residue

33.3 Weights will be routinely reported for controlled substances and marijuana.

See the UOM section for guidance. Until the UOM policy is implemented, simply truncate the last decimal place prior to reporting.

33.3.1 Total weights that have been approximated will be reported as "approximate total weight".

33.3.2 Net weights will not include any packaging.

33.3.3 Gross weights will include innermost packaging and be clearly delineated when they are reported.

33.4 The following are examples of report wording for typical drug items using the Administrative Sampling Plan.

33.4.1 Simple possession

33.4.1.1 Items with one specimen

- Marijuana, 5.1 grams (0.17 ounce) of plant material including innermost packaging.
- 0.501 gram of solid material including innermost packaging, found to contain Heroin (Schedule I).
- 0.254 gram of solid material, found to contain Heroin (Schedule I).
- Tablet, found to contain Oxycodone (Schedule II) and Acetaminophen.
- The tablet was found to contain Oxycodone (Schedule II) and Acetaminophen.

33.4.1.2 Items with more than one specimen

- Total weight: 5.1 grams (0.17 ounce) of plant material including innermost packaging. The contents of one were analyzed and found to contain Marijuana.
- Total weight: 0.95 gram of solid material including innermost packaging. The contents of one were analyzed and found to contain Cocaine (Schedule II)

- The contents of one were analyzed and found to contain Cocaine (Schedule II); net weight of the one: 0.95 gram of solid material. The gross weight of the remainder was 2.33 grams(s) including innermost packaging.

33.4.2 Possession with intent to distribute or distribution

33.4.2.1 Five specimens or less

- The contents of three were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the three: 0.476 gram of powder.
- The contents of two were analyzed separately and each was found to contain Marijuana; total net weight of the two: 16.3 grams (0.57 ounce) of plant material.
- Total weight: 6.3 grams (0.22 ounce) of plant material including innermost packaging. The contents of four were analyzed separately and each was found to contain Marijuana.

33.4.2.2 More than five specimens

- The contents of five were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the five: 0.473 gram of powder. The gross weight of the remainder was 2.331 gram(s) including innermost packaging.
- The contents of five were analyzed separately and each was found to contain Marijuana; total net weight of the five: 16.3 grams (0.57 ounce) of plant material. The gross weight of the remainder was 12.3 gram(s) including innermost packaging.
- Total weight: 6.3 grams (0.22 ounce) of plant material including innermost packaging. The contents of five were analyzed separately and each was found to contain Marijuana.

33.4.2.3 More than five specimens, meeting a weight threshold

- The contents of eight were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the eight: 30.5 grams of solid material. The gross weight of the remainder was 12.3 gram(s) including innermost packaging.
- The contents of six were analyzed separately and each was found to contain Marijuana; total net weight of the six: 16.34 grams (0.57 ounce) of plant material. The gross weight of the remainder was 12.37 gram(s) including innermost packaging.

33.5 The following are examples of report wording for typical drug items using the Hypergeometric Sampling Plan.

- The contents of fourteen additional bags were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the fourteen: 1.312 grams of powder. (resubmissions)
- The contents of twenty-nine bags were analyzed separately and each was found to contain Cocaine (Schedule II); total net weight of the twenty-nine: 2.427 grams of powder. The gross weight of the remainder was 12.332 gram(s) including innermost packaging. (initial submissions)

33.6 For substances in Schedule V – VI involving misdemeanor prosecutions or non-controlled substances, tablets and capsules visually examined using only pharmaceutical identifiers will be reported following these two examples.

33.6.1 "Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with Zoloft, a Schedule VI pharmaceutical preparation containing Sertraline. There was no apparent tampering of the dosage units and no further tests are being conducted."

33.6.2 "Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule VI pharmaceutical preparation containing Sertraline. There was no apparent tampering of the dosage units and no further tests are being conducted."

33.7 For substances and preparations in Schedules IV and above, tablets and capsules which were representatively sampled will be reported following these two examples.

33.7.1 "One dosage unit was analyzed and found to contain Diazepam, Schedule IV. Visual examination of the physical characteristics, including shape, color and manufacturer's markings of both the analyzed and remaining dosage units, was consistent with Valium, a pharmaceutical preparation containing Diazepam. There was no apparent tampering of the dosage units."

33.7.2 "One dosage unit was analyzed and found to contain Diazepam, Schedule IV. Visual examination of the physical characteristics, including shape, color and manufacturer's markings of both the analyzed and remaining dosage units, was consistent with a pharmaceutical preparation containing Diazepam. There was no apparent tampering of the dosage units."

33.8 For tablets and capsules with substances and preparations in Schedules IV and above, where the analytical results are inconsistent with the manufacturer's specifications with regard to content, report following these two examples.

33.8.1 "One dosage unit was analyzed and found to contain Acetaminophen (non-controlled). Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule III pharmaceutical preparation containing Hydrocodone and Acetaminophen; therefore, the contents were inconsistent with dosage unit labeling."

33.8.2 "Five dosage units were analyzed separately and each was found to contain Diazepam (Schedule IV). Visual examination of the physical characteristics, including shape, color and manufacturer's markings, was consistent with a Schedule IV pharmaceutical preparation containing Alprazolam; therefore, the contents were inconsistent with dosage unit labeling."

33.9 "No controlled substances found" or "No controlled substances identified" will be used to describe items where no compounds were found or identified that are controlled substances.

33.10 "Not analyzed" will be used for items not examined chemically or visually.

33.11 "Insufficient for identification" will be used to describe items with too little sample for a complete identification.

33.12 In cases where items have been analyzed for possible tampering or substitution, the results will have an additional statement of either "Meets label specifications" or "Does not meet label specifications". Additional clarifying wording should be used such as "Does not meet label specifications with regard to concentration" at the discretion of the Section Supervisor.

33.13 Quantitation results should be truncated to a whole integer following these three examples:

33.13.1 25.93 grams of solid material, found to contain Cocaine (Schedule II), 35% pure.

33.13.2 12.523 grams of powder, found to contain Cocaine Hydrochloride (Schedule II), 45% pure.

33.13.3 Hashish oil (Schedule I), found to contain 34% tetrahydrocannabinol by weight.

33.14 Resubmissions for cocaine salt/base analysis will be reported as per the following examples:

33.14.1 "The contents of the previously examined five plastic bag corners were analyzed separately and each was found to contain Cocaine base (Schedule II)."

33.14.2 "The contents of the previously examined three ziplock bags were analyzed separately and each was found to contain Cocaine Hydrochloride (Schedule II)."

33.14.3 Solid material, found to contain Cocaine base (Schedule II).

33.14.4 The solid material was found to contain Cocaine base (Schedule II).

33.15 Substances listed as precursors in Code of Virginia §18.2-248(J) or are defined in §18.2-248(K) shall be reported as such as per the following examples:

33.15.1 Tablet, found to contain Pseudoephedrine (a listed substance in §18.2-248(J)).

33.15.2 Liquid, found to contain Ephedrine/Pseudoephedrine (a listed substance in §18.2-248(J)).

33.16 Synthetic cannabinoids will be reported as per the following examples:

33.16.1 When a Listed Synthetic Cannabinoid is present: "0.254 gram of powder, found to contain 1-pentyl-3-(1-naphthoyl)indole (JWH-018) (a synthetic cannabinoid listed in § 18.2-248.1:1)."

33.16.2 When a compound within a defined structural class is present (but not specifically listed): "0.254 gram of plant material, found to contain 1-propyl-2-methyl-3-(1-naphthoyl)indole (JWH-015). This compound is a synthetic cannabinoid as defined in § 18.2-248.1:1(A)(1)(b) and is within the structural class 3-(1-naphthoyl)indole."

33.16.3 When a Non-Listed/Non-Structural Class Synthetic Cannabinoid may be present, but is not identified: "No controlled substances or synthetic cannabinoids defined in § 18.2-248.1:1 were identified."

33.16.4 When a mixture of compounds is present (where one is within a defined structural class but not specifically listed along with a listed compound): "0.254 gram of plant material, found to contain a mixture of synthetic cannabinoids: 1-pentyl-3-(1-naphthoyl)indole (JWH-018) (a synthetic cannabinoid listed in § 18.2-248.1:1) and 1-propyl-2-methyl-3-(1-naphthoyl)indole (JWH-015), a synthetic cannabinoid as defined in § 18.2-248.1:1(A)(1)(b) and is within the structural class 3-(1-naphthoyl)indole."

33.17 Isomers of substances listed in Schedule I will be reported as per the following examples:

33.17.1 Option 1 (the isomers can be analytically distinguished): "3-Fluoromethamphetamine (Schedule I), an isomer of 4-fluoromethamphetamine."

33.17.2 Option 2 (the isomers have not been analytically distinguished): "4-Fluoromethamphetamine or one of its isomers as defined in §54.1-3446(3) (Schedule I)."

33.18 Different reporting options consistent with applicable laws may be used at the discretion of the Section Supervisor.

34 DRUG REVERSALS

34.1 Introduction

The Department will assist law enforcement agencies with preparation of materials to be used in drug reversals, buy/bust scenarios, internal security setup operations and "show and tell" drugs. In all instances, the requesting agency must assume full responsibility for distribution of these materials.

34.2 Procedure

34.2.1 The agency must make a written request on the Agency's letterhead detailing the scope of the operation, materials needed and projected timeframes. This request must be approved by the Section Supervisor or Chemistry Program Manager and Laboratory Director.

34.2.2 Any controlled substances must be provided by the requesting agency or a cooperative neighboring jurisdiction.

34.2.2.1 If the material is from a case previously analyzed by the Department, this must be made clear by the agency.

34.2.3 Cutting materials must be supplied by the requesting agency and will be subject to approval by the Section Supervisor.

34.2.3.1 The Department may provide substances added to the material for "marking" purposes. Examples of such substances are benzocaine, lidocaine and diphenhydramine.

34.2.3.2 There may be times when uncut materials are required. However, whenever possible, the controlled substances should be diluted to approximately 1% drug by weight to allow for future analysis while enhancing the safety of the operation. This will be evaluated on a case by case basis.

34.2.4 All packaging material must be supplied by the requesting agency.

34.2.4.1 Materials will be packaged at the laboratory in accordance with the agency's request.

34.2.4.2 Packages must allow for future testing of the material when it is re-submitted.

34.2.5 An authentic sample of the final preparation will be kept by the analyst for future comparison. All analytical data such as weights, composition, notes, chromatograms and spectra are to remain with the authentic sample.

34.2.5.1 Samples and supporting analytical data shall be maintained for at least six years from the date of preparation.

34.2.6 The requesting agency must submit all cases resulting from a particular operation to the attention of the analyst who prepared the material. These should be clearly marked on the RFLE.

34.2.7 The Department assumes no responsibility or liability for the security of these materials once the law enforcement agency takes possession of them.

34.2.8 The Department assumes no responsibility or liability for any use of these materials by the requesting agency or any other person.

35 QUALITY ASSURANCE

35.1 Introduction

35.1.1 The purpose of this section is to provide a uniform Quality Assurance Program for the Controlled Substances Section of the Virginia Department of Forensic Science. It is to establish a baseline or reference point of reliability and system performance.

35.1.2 It is expected that the analyst will report any unacceptable or anomalous behavior of any of our analytical systems immediately to either their Section Supervisor or the appropriate Instrument Specialist (Primary Operator). It is further expected that appropriate steps which ensure resolution of the issue will follow ASAP and be properly documented.

35.2 Reagents

35.2.1 Chemicals and solvents used in qualitative reagents should be of at least ACS reagent grade.

35.2.2 Solvents used to dissolve samples or standards should be a high quality, low residue solvent (e.g., HPLC grade, OMNISOLV, OPTIMA).

35.2.3 Water used in reagent preparation should be either deionized (DI) or reverse osmosis (RO).

35.2.4 Stock solutions of general color test reagents and TLC sprays will be made up as needed. The Department *Reagent Worksheet* shall be used to record reagent preparation. After they are made, they will be checked with the corresponding primary or secondary standard listed below in Table 3 and results, date and initials will be recorded.

35.2.5 Color test reagents and TLC sprays will be verified every three months during the shelf life of the reagent (2 years unless otherwise noted). The *Reagent QA Check Worksheet* shall be used for this purpose.

35.2.6 Individual chemists may have unique reagents other than the ones listed in Table 3 and it will be their responsibility to check them with an appropriate standard and document accordingly. For single use reagents, this documentation may be in the case file.

TABLE 3: Common Reagents and Appropriate Check Compounds

REAGENT	CHECK COMPOUND
Duquenois	Marijuana
Marquis	Heroin
Froehdes	Heroin
Meckes	Heroin
Cobalt Thiocyanate	Cocaine
Ehrlichs	LSD
Iodoplatinate	Cocaine
Iodoplatinate/Ceric Sulfate	Caffeine
$KMnO_4$	Acetaminophen
Fast Blue B and Fast Blue BB	Hash Oil or Marijuana

35.3 Standards

35.3.1 Primary and quantitative reference standards should normally be at least of United States Pharmacopeia – National Formulary (USP-NF) quality. This applies to both powders purchased from a manufacturer/supplier and pharmaceutical preparations.

35.3.1.1 Receipt, storage and use of controlled drug standards shall be recorded and records maintained as required by § 54.1-3404.

35.3.1.2 Standards used as reference materials in casework are considered critical supplies and shall be purchased from manufacturers approved by the Chemistry Program Manager.

35.3.1.2.1 The following manufacturers/vendors/suppliers are pre-approved for the purchase of new reference standards:

- USP
- Alltech Associates (Grace – Discovery Sciences)
- Cerilliant
- Sigma-Aldrich and its subsidiaries
- Steraloids, Inc.
- Cayman Chemicals
- Lipomed

35.3.1.2.2 Pharmaceutical preparations may be purchased from any licensed pharmacy or the patented drug manufacturer for use as reference materials.

35.3.1.3 Primary reference standards are those purchased from a reputable manufacturer.

35.3.1.4 Secondary standards are those which are obtained or synthesized within the laboratory. These may be from previously analyzed case samples.

35.3.1.5 Quantitative standards have a known purity, known accuracy and are purchased from a reputable manufacturer including those listed in ¶ 35.3.1.2.1.

35.3.2 For all standards obtained for drug lab use, a qualified examiner will be responsible for obtaining a mass spectrum, IR or other suitable definitive instrumental data (data provided by the manufacturer is not sufficient, although it should be stored with lab generated data). The hard copy data will be filed. The hard copy should include the following data:

- Lot# or log book code
- Standard name
- Concentration, as appropriate
- Amount injected (for MS only)
- Analyst's initials and date

35.3.3 After the examiner gathers the data and insures that it agrees with known published spectra or that the data is consistent with the unique compound represented from both a chemical and data interpretation perspective, label the standard bottle with "MS", "IR", etc., the date and initials. Include the source of the standard and record all pertinent information in the Standards File.

35.3.4 If an examiner needs a standard from a new lot that has not been documented in this fashion, the examiner must perform the above procedure prior to using it for drug case work.

35.3.5 When positive results are achieved in casework, the corresponding standard(s) must be properly documented in the case file. Standards used for TLC should be documented in the case notes, whereas standards used for instrumental methods may be documented either in the case notes or on the data. If the same standard is used for both types of tests, it only needs to be documented once in the case file.

35.4 Balances

35.4.1 All analytical and toploading balances will be checked weekly for accuracy using Class S-1 weights or better. Record the weights in the log book with the date and initials.

35.4.2 All high capacity balances will be checked monthly for accuracy using Class S-1 or Class F weights or better. Record the weights in the log book with the date and initials.

35.4.3 Balances shall be calibrated by an outside vendor annually.

35.4.4 Weights used to check balance accuracy shall be sent to vendor for re-certification every three years.

35.4.5 The balances listed below in Table 4 represent examples of a balance class or type along with the corresponding check weights. If a balance does not fit into these categories, use three weights within its range as approved by the Chemistry Program Manager.

35.4.5.1 Place weigh paper or boat on balance, tare and add weight.

35.4.5.2 If a result from the performance check is outside of the acceptable range, first ensure that the balance is level and clean prior to rechecking.

35.4.5.3 If applicable, use the internal calibration function of the balance prior to rechecking.

35.4.5.4 If a result is outside of the acceptable range after performing the actions found in 35.4.5.2 and 35.4.5.3, the balance shall be immediately taken out of service until maintenance and/or calibration are performed by an approved vendor.

TABLE 4: Balances and Appropriate Check Weights

BALANCE TYPE	BALANCE EXAMPLES	CHECK WEIGHTS
Analytical (dual range)	Mettler XS 105	0.00100 (± 0.00005) gram, 20.00000 (± 0.00005) grams 50.0000 (± 0.0005) grams 100.0000 (± 0.0005) grams
Analytical	Mettler AE 160 Sartorius Basic	0.0100 (± 0.0002) gram, 1.0000 (± 0.0003) gram, 20.0000 (± 0.0005) grams
Toploading (± 0.01) gram	Mettler PE 2000 Mettler PE 1600 Mettler PB302 Ohaus Port-O-Gram Sartorius 2100	1.00 (± 0.02) gram, 10.00 (± 0.05) grams, 100.00 (± 0.05) grams
Toploading (± 0.001) gram	Ohaus Explorer Mettler PB303	0.100 (± 0.002) gram 1.000 (± 0.002) gram 100.000 (± 0.005) grams
High Capacity (g)	A.N.D. Electronic (1000, 10,000, 30,000 g) Ohaus CQ10R33 (100, 1000, 10,000 g)	100 (± 1) grams 1000 (± 1) grams 10,000 (± 5) grams 30,000 (± 10) grams
High Capacity (kg)	Ohaus DS5-M (1 or 2, 10, 20kg) Ohaus DS10-L (1 or 2, 10, 30 kg) Ohaus ES50L (1 or 2, 10, 30 kg)	1.00 or 2.00 (± 0.02) kilograms 10.00 (± 0.02) kilograms 20.00 (± 0.02) kilograms 30.00 (±0.02) kilograms

35.4.6 Accuracy and precision must be established after a balance has been put into service after purchase or repair. The *Balance Accuracy and Precision Worksheet* should be used for this purpose.

35.4.6.1 The check weights listed in Table 4 are weighed and recorded five times.

35.4.6.2 The mean and % relative standard deviation (%RSD) are calculated for each weight.

%RSD = 100 * (standard deviation / mean)

35.4.6.3 Acceptance Criteria:

35.4.6.3.1 The accuracy of each weight should meet the criteria in Table 4.

35.4.6.3.2 %RSD must be less than or equal to 5 %.

35.4.6.3.3 The balance will be immediately taken out of service if these criteria are not met.

35.5 Thin Layer Chromatography

35.5.1 TLC bath solutions will be made up as needed. After they are made, they will be checked with the compound(s) listed below in Table 5 and results, date and initials will be recorded in the logbook. Limited use baths not listed in Table 5 will be checked by running appropriate standards along with the sample(s).

35.5.2 The baths should be refreshed daily.

35.5.3 Day-to-day performance is checked by running the standard along with the sample(s).

TABLE 5: TLC Baths and Check Compounds

REAGENT	CHECK COMPOUNDS
TLC1 (9:1)	Cocaine/Heroin
TLC2 (18:1)	Cocaine/Heroin
TLC3 (T-1)	Cocaine/Heroin
TLC4 / TLC5	Marijuana

35.6 Gas Chromatographs

35.6.1 Record any maintenance performed in the logbook, date and initial.

35.6.2 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

35.6.3 Weekly

35.6.3.1 Change all septa (unless a Merlin Microseal is installed).

35.6.3.2 The column performance is checked by injecting a standard with the sample(s).

35.6.4 Monthly

35.6.4.1 Run a mixture of DFTPP, Methamphetamine, Cocaine, Tetracaine and Heroin standards. The concentration of these standards should be 2 mg/mL or less. Method conditions should mimic those used in a general screen run by examiners.

35.6.4.1.1 Record in log book, date and initial. The chromatogram should demonstrate good chromatographic performance. Acceptable results must be documented in either the logbook or on the data.

35.6.4.1.2 Any performance discrepancies or degradation must be reported immediately to a supervisor.

35.6.4.1.3 Store hardcopy of data for approximately one year.

35.6.4.2 Archive data files, sequence files and sequence log files to suitable long-term storage media. Retain for at least six years.

35.6.5 Every 3 Months

35.6.5.1 Change injection port liners as needed.

35.6.5.2 Clean FID detectors. Low usage FID detectors will be cleaned only as needed.

35.6.6 Semi-annually

35.6.6.1 Archive important non-data files to suitable long-term storage media, including macros and methods. Retain for at least six years.

35.6.7 Yearly

35.6.7.1 Remove columns, clean injection ports and FID detectors. Reinstall or replace columns as needed.

35.6.7.2 Replace the split vent traps for Agilent 6890 and higher series gas chromatographs.

35.6.8 Placement of Instrument into Service

35.6.8.1 After significant maintenance has been performed, run the standard mixture as outlined in ¶ 35.6.4.1.

35.6.8.2 New instrument installation

35.6.8.2.1 Obtain documentation from the instrument service representative which demonstrates that the instrument performs to manufacturer's specifications.

35.6.8.2.2 Load/modify appropriate macros and test functionality.

35.6.8.2.3 After methods are created, run a standard on a representative sample of the methods (e.g., low temp., mid temp., high temp., screen methods) to demonstrate efficacy.

35.6.8.2.4 Run the standard mixture as outlined in ¶ 35.6.4.1 ten times to demonstrate chromatographic reproducibility.

35.6.8.2.5 Archive methods and data analysis macros to suitable long-term storage media.

35.6.8.2.6 A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.

35.6.8.2.7 Retain instrument verification documentation.

35.7 Mass Spectrometers

35.7.1 Record any maintenance performed in the logbook, date and initial.

35.7.2 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

35.7.3 Daily

- Autotune - Use "Reset to Defaults", if available, and "Autotune" for GC/MS systems for routine "seized" drug analysis. Quicktune or its equivalent may be used as determined by the primary

operator. Specialized tunes may be used on a case by case basis as determined by the primary operator and the examiner in concert with the Chemistry Program Manager.

Table 6: Autotune Acceptance Criteria

UNE PARAMETER	SPECIFIC PARAMETER	ACCEPTANCE RANGE
Peak widths	0.50 Da	± 0.05 Da spread between values ≤ 0.05)
Mass assignment	69.00, 219.00, 502.00 Da	± 0.10 Da
Isotope Ratios	Ratio of mass 70 to 69	0.5 – 1.6%
Isotope Ratios	Ratio of mass 220 to 219	3.2 – 5.4%
Isotope Ratios	Ratio of mass 503 to 502	7.9– 12.3%

- An acceptable tune will be noted in the logbook with the date and initials of the approving examiner.
- Run Background and note in logbook with the date and initials.
- Retain a copy of the daily autotune report and background for 6 years.
- Change septum or perform maintenance on "Merlin Microseal", if needed.
- If GC/MS is used for GC retention time data, and this data is not stored in an appropriate case file, run standards as needed and retain hardcopy for lab files. Lab standards must be retrievable and maintained for the same time period as the case files. They may be kept in the laboratory until the case files are sent to archives. At that time the lab standard files must be archived along with the case files.

35.7.4 Weekly

- Run a mixture of Methamphetamine, DFTPP, Cocaine, Tetracaine and Heroin reference standards and store representative peaks and spectra in file. Concentration should be 2 mg/mL or less. Method conditions should mimic those used in a general screen run by examiners. The chromatogram should demonstrate good chromatographic performance. Acceptable results must be documented in the logbook. Retain a copy for at least one year.
- Check Helium and other gas supplies, replace as needed.
- Replace injection port liner as needed.

35.7.5 Semi-monthly

- Archive data files, sequence files and sequence log files to suitable long-term storage media. Retain for at least six years.

35.7.6 Monthly

- Check PFTBA level and do full source clean, if needed.
- Check mechanical pumps oil level.
- Clean injection port if needed.

35.7.7 Semi-annually

- Archive important non-data files to suitable long-term storage media. Include macros, methods and user libraries for Agilent systems. Retain for at least six years.

35.7.8 Yearly

- Replace GC column with a new one.
- Schedule preventive maintenance as required.

35.7.9 Placement of Instrument into Service

35.7.9.1 After significant maintenance has been performed

35.7.9.1.1 Tune and run background, as necessary, as outlined in section 34.7.2.

35.7.9.1.2 Run the standard mixture as outlined in section 34.7.3.

35.7.9.2 New instrument installation

35.7.9.2.1 Obtain documentation from the instrument service representative which demonstrates that the instrument performs to manufacturer's specifications.

35.7.9.2.2 Run blanks with the threshold set to various values, beginning with zero, to determine the proper mass detect threshold setting for the instrument.

35.7.9.2.3 Load/modify appropriate macros and test functionality.

35.7.9.2.4 After methods are created, run a standard on a representative sample of the methods (e.g., low temp., mid temp., high temp., screen methods) to demonstrate efficacy.

35.7.9.2.5 Run either the QA mixture or a cocaine standard ten times to demonstrate chromatographic reproducibility.

35.7.9.2.6 Load applicable user libraries.

35.7.9.2.7 Archive methods and data analysis macros to suitable long-term storage media.

35.7.9.2.8 A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.

35.7.9.2.9 Retain instrument verification documentation.

35.8 HP 1100 LC/MS

This instrument is now in the Toxicology Section.

35.9 Support HP 5973 GC/MS

35.9.1 Prior to collecting data for any analysis, as needed, the manufacturer's "freshtune" and "autotune"* programs must be run. Specialized tunes may be used as determined by the primary operator. A background run shall also be done.

35.9.2 Retain a copy of the autotune and background for 6 years. An acceptable tune will be noted in the logbook with the date and initials of the approving examiner.

35.9.3 Record all maintenance performed in a logbook, date and initial.

35.9.4 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

35.9.5 Methamphetamine, DFTPP, Cocaine, Tetracaine and Heroin reference standards, in a suitable concentration for this instrument, should be run after installation of a new or cleaned injection port liner.

35.9.6 The instrument shall be maintained to manufacturer's specifications, using repair/replacement guidelines set forth by the manufacturer, or to maintain optimum operating conditions.

35.9.7 Data/Method files Archival

35.9.7.1 Data files shall be archived to suitable long-term storage media on a monthly basis and retained for at least six years. Method files shall be archived semi-annually.

35.10 FTIR

35.10.1 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

35.10.2 Daily

35.10.2.1 The throughput of the bench will be documented daily. Both the maximum and minimum values of the interferogram, the location of the centerburst, and the gain setting will be recorded in the log book, dated and initialed.

35.10.2.2 Record all maintenance performed in a logbook, date and initial.

35.10.2.3 The location of the centerburst must be stable at 1024 ± 16, if not, then align the bench and perform maintenance if needed.

35.10.2.4 If the sum (absolute value) of the maximum and minimum is less than 8.0 at a gain of 1.0, then align the bench and perform maintenance if needed.

35.10.2.5 The OMNIC software checks a number of different performance characteristics including collecting a polystyrene spectrum and checking the peak-to-peak noise in its performance validation package (e.g., VAL-Q, VAL-PRO). The performance check will be run daily. If the instrument does not pass any of the acceptance criteria, perform maintenance as needed. Record successful results in the logbook, date and initial. A hardcopy of the report will be generated and stored in the laboratory for six years.

35.10.2.6 An uncorrected standard of procaine hydrochloride will be run on the ATR accessory and searched against a library containing uncorrected standards collected using the ATR accessory. The match should be 95% or greater. Record in logbook, date and initial. A hardcopy of the standard and library match will be generated and stored in the laboratory for six years.

35.10.2.7 A record of all samples will be kept in a logbook which includes the date, FS Lab# and/or file name, and the initials of the user.

35.10.3 Weekly

35.10.3.1 The bench will be aligned weekly using the "Align Bench..." function and the throughput values will be documented following the same criteria as listed above.

35.10.3.2 The throughput of the GC/lightpipe will be documented weekly. The oven temperature will be set to 200° C, the lightpipe temperature will be set to 270° C, and the transfer line temperature will be set to 270° C. Both the maximum and minimum values of the interferogram, the location of the centerburst, the gain setting will be recorded in the log book, dated and initialed. If the sum (absolute value) of the maximum and minimum energy is less than 8.0 at a gain of 1.0, then perform maintenance as needed.

35.10.3.3 The noise level of the GC/lightpipe will be checked weekly. A spectrum will be taken while the GC/lightpipe is in the same configuration as the throughput check, taking 128 scans with the resolution set to 8 cm^{-1} and the gain set to 1.0. Select the 2200-2100 cm^{-1} range and measure the noise using "Analyze" then "Noise". The Peak-to-Peak noise value should be below 0.10, if not, perform maintenance as needed. Record in logbook, date and initial.

35.10.4 Monthly

35.10.4.1 Archive data files to suitable long-term storage media. Retain for at least six years.

35.10.5 Placement of Instrument into Service

35.10.5.1 After significant maintenance has been performed, run the daily and weekly QA as outlined above.

35.10.5.2 New instrument installation

35.10.5.2.1 Obtain documentation from the instrument service representative which demonstrates that the instrument performs to manufacturer's specifications.

35.10.5.2.2 After experiments are created, run a cocaine base and a cocaine hydrochloride standard on each to demonstrate efficacy. An additional standard of procaine hydrochloride must be run on methods utilizing the ATR attachment.

35.10.5.2.3 Run a cocaine standard ten times to demonstrate reproducibility.

35.10.5.2.4 Archive experiments and macros to suitable long-term storage media.

35.10.5.2.5 A summary of the verification shall be sent to the Chemistry Program Manager for approval prior to placing a new instrument into service.

35.10.5.2.6 Retain instrument verification documentation.

35.11 AccuTOF-DART

35.11.1 Record any maintenance performed in the logbook, date and initial.

35.11.2 The functionality of new macros shall be verified by the instrument operator prior to use in casework and documented in the corresponding instrument logbook.

35.11.3 Daily

35.11.3.1 Start DART and Mass Center software, and turn on DART gases and power supply.

35.11.3.2 Check size of current Mass Center project. Open new project, if needed.

35.11.3.3 Turn on DART gas temperature control, load AccuTOF tune file, put AccuTOF in "operate" mode and allow five minutes to equilibrate.

35.11.3.4 Using the DART_POS_SWITCH_02 method, acquire PEG600, drift compensation/positive control mix (cocaine, methamphetamine and nefazodone) and methyl stearate spectra.

35.11.3.5 Perform calibration and check methyl stearate (30V function).

35.11.3.5.1 Generate and save a PEG600 internal mass calibration file.

35.11.3.5.2 Generate and save an internal mass drift compensation file on the protonated molecule of cocaine (304.1549 Da).

35.11.3.5.3 Acceptance criteria for methamphetamine and nefazodone positive controls: the $[M+H]^+$ peaks shall be within ± 5 mmu of the calculated protonated molecules at 150.1283 Da and 470.2323 Da, respectively.

35.11.3.5.4 Using internal mass calibration and internal mass drift compensation produce an averaged, background subtracted, centroided spectrum of methyl stearate.

35.11.3.5.5 Acceptance Criterion: The $[M+H]^+$ peak is within ±3 mmu of the calculated protonated molecule at 299.2950 Da.

35.11.3.6 Store hardcopy of daily calibration for at least 6 years.

35.11.4 Weekly

35.11.4.1 Check rough pump oil level and siphon oil back into pump from mist filter.

35.11.4.2 Close all open software and defragment hard drive.

35.11.4.3 Check helium and other gas supplies and replace as needed.

35.11.5 Monthly

35.11.5.1 Clean ion guide components of AccuTOF, as needed, to maintain performance.

35.11.5.2 If ion guide is cleaned, after pumping down system and conditioning the MCP detector, attach electrospray ionization source and infuse 100 ppb reserpine in methanol to check resolution and intensity of reserpine $[M+H]^+$.

35.11.5.2.1 Adjust AccuTOF settings as needed, and save tune file. Print screen shot of "Spectrum Monitor" showing resolution and intensity achieved.

35.11.5.2.2 Reset the PEG+H global calibration file.

35.11.5.2.3 Resave method tune files with appropriate Orifice1 voltages.

35.11.5.3 Archive data files and spectral libraries to suitable long-term storage media and retain for at least 6 years.

35.11.6 Annually

35.11.6.1 Schedule preventative maintenance for AccuTOF-DART system, as required.

35.11.6.2 Perform maintenance on nitrogen generator, as required.

35.12 Refrigerators/Freezers

35.12.1 The temperature of refrigerators and freezers which store reagents, standards or evidentiary material should be checked and recorded on a weekly basis. The *CS Refrigerator Temperature Log* and the *CS Freezer Temperature Log* should be used for this purpose.

35.12.2 For refrigerators, the temperature shall be between 2 – 8 ° C.

35.12.3 For freezers, the temperature shall be below -5 ° C.

35.12.4 If temperatures fall outside the range, the thermostat should be adjusted. If necessary, the contents of the refrigerator or freezer should be moved to another refrigerator or freezer.

35.12.4.1 Critical reagents and standards should be re-verified if the temperature in the refrigerator exceeds 15 ° C or the freezer exceeds 0 ° C prior to use in case work.

35.12.5 Retain temperature logs for at least 6 years.

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See Training Manual for additional references

37 SCHEDULING

37.1 Schedule I

37.1.1 High potential for abuse with no legal medical use in the U.S.

37.1.2 Examples include: Heroin, MDA, LSD, mescaline, peyote, hashish oil with more than 12% THC, psilocybin, and psilocyn

37.2 Schedule II

37.2.1 High potential for abuse, have some medical use, use may lead to severe dependence

37.2.2 Examples include: Cocaine, opium, morphine, codeine, oxycodone, PCP, methamphetamine, amphetamine, amobarbital, secobarbital, and pentobarbital

37.3 Schedule III

37.3.1 Lower potential for abuse than Schedule II substances, have a medical use, cause some dependence

37.3.2 Examples include: Most barbiturates, some codeine preparations, ketamine, phendimetrazine, and anabolic steroids

37.4 Schedule IV

37.4.1 Low potential for abuse, have a medical use, can cause low dependence

37.4.2 Examples include: Barbital, diazepam, meprobamate, phenobarbital, chlordiazepoxide, pentazocine, and benzodiazepines

37.5 Schedule V

37.5.1 Similar to Schedule IV but less potential for abuse and less dependence

37.5.2 Examples include: Low concentrations of codeine, ethylmorphine or opium, and numerous cough syrups

37.6 Schedule VI

37.6.1 All other drugs requiring a prescription

37.6.2 Examples include: Antibiotics, antihistamines, tricyclic antidepressants

37.7 If there is any question as to the scheduling of a particular substance, refer to the current Code of Virginia. If any question still remains, report only the identity of the material and leave the scheduling to the court system.

38 WEIGHT THRESHOLDS

38.1 Any Schedule I or II Controlled Substance:

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth

38.2 Coca Leaves:

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
500 grams	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
10 kilograms	§ 18.2-248: distribution or possession with intent to distribute

38.3 Cocaine:

1 ounce (28.35 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture) § 18.2-248.01: transportation of controlled substances into Commonwealth
½ pound (226.8 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture)
500 grams	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
10 kilograms	§ 18.2-248: distribution or possession with intent to distribute

38.4 Cocaine Base:

1 ounce (28.35 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture) § 18.2-248.01: transportation of controlled substances into Commonwealth
½ pound (226.8 grams)	Sentencing guidelines- cocaine (PWID, distribution, manufacture)
250 grams	§ 18.2-248: distribution or possession with intent to distribute
2.5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute

38.5 Ecgonine:

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
500 grams	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute
10 kilograms	§ 18.2-248: distribution or possession with intent to distribute

38.6 Heroin:

1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
100 grams	§ 18.2-248: distribution or possession with intent to distribute
1 kilogram	§ 18.2-248: distribution or possession with intent to distribute
5 kilograms	§ 18.2-248: distribution or possession with intent to distribute

38.7 Marijuana:

½ ounce	§ 18.2-248.1: marijuana distribution § 18.2-255.2: sale of drugs on or near certain properties
1 ounce	§ 18.2-255: distribution to persons under 18
1 pound	§ 18.2-308.4: possession of firearms and certain Controlled Substances
5 pounds	§ 18.2-248.01: transportation of controlled substances into Commonwealth § 18.2-248.1 marijuana distribution
100 kilograms	§ 18.2-248: distribution or possession with intent to distribute
250 kilograms	§ 18.2-248: distribution or possession with intent to distribute

38.8 Methamphetamine (methamphetamine, its salts, isomers, or salts of its isomers)*:

10 grams	§ 18.2-248: distribution or possession with intent to distribute
1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
100 grams	§ 18.2-248: distribution or possession with intent to distribute
250 grams	§ 18.2-248: distribution or possession with intent to distribute

38.9 Methamphetamine (a mixture or substance containing a detectable amount):

20 grams	§ 18.2-248: distribution or possession with intent to distribute
28 grams	§ 18.2-248.03: Manufacturing, distribution or intent to manufacture or distribute
1 ounce	§ 18.2-248.01: transportation of controlled substances into Commonwealth
200 grams	§ 18.2-248: distribution or possession with intent to distribute
227 grams	§ 18.2-248.03: Manufacturing, distribution or intent to manufacture or distribute
1 kilogram	§ 18.2-248: distribution or possession with intent to distribute

(*requires quantitation)

39 HYPERGEOMETRIC TABLE

Population (N)	Failure%=10%, Confidence Level=95%	Population (N)	Failure%=10%, Confidence Level=95%
1 – 10	ALL	40	21
11	9	41	18
12	9	42	18
13	10	43	19
14	11	44	19
15	12	45	20
16	12	46	20
17	13	47	21
18	14	48	21
19	15	49	22
20	16	50	22
21	13	60	23
22	14	70	24
23	14	80	24
24	15	90	25
25	16	100	25
26	16	200	27
27	17	300	28
28	18	400	28
29	18	500	28
30	19	600	28
31	16	700	28
32	17	800	28
33	17	1000	29
34	18		
35	18		
36	19		
37	19		
38	20		
39	20		

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40 COMMONLY USED ABBREVIATIONS

The following is a list of abbreviations commonly used by examiners in the Controlled Substances Section. This list has been generated to assist in the interpretation of case file notes and is not a standardized list of required abbreviations. The abbreviations are appropriate written in either lower or upper case and they are appropriate with or without punctuation such as periods. Common chemical formulas, chemical, mathematical and shorthand abbreviations are equally acceptable and will not be listed here.

Definitions	Abbreviations
Alexandria	Alex
Arlington County	ARL
autosampler vial	ASV
Background	bkg
Bag	b, bg
Because	bc
Black	bl
Blank	blk
blotter paper	blot pap, blp
Bottle	btl, bot
brought sample back up in solvent	↑
Brown	brn
Burnt residue	br
Canister	cn
Capsule	cap, cp, cps
Cellophane	cel, cell, cello
Charred	chr, char
Cigar	cgr
cigarette	cig
Clear	clr
color test(s)	ct
commercial	com
Concentrated	Conc
Container	Cont
Containing	con, c/, =>, ©, c (with a line over it), →
Continued	Cont'd
Corner	C
cross contamination	x-cont, x-contam
Crystalline	cryst, xtalline
cystolithic hairs	Cyst. ch, cyst hair
Dark	Dk
Development	devel
Device	dev
Diluted	dil
dimethyl terephthalate	DMTP
dried down sample	↓
Effervescence	eff, effer, efferv
Empty	MT

Envelope	env
Evidence	ev, evid, evd, e
Extract	ext
Extraction blank	EB
Fairfax County	FFX
Fingerprints	LX
fragment(s)	frag
Glass	gl
Glassine	glas, gla
Green	gr, grn
hand made	hdm
hand rolled	hr
Heat	Δ, H
Imprinted	imp
Individual	ind
Insert (for weak samples in ASV)	INS
Instrumental Data for Drug Analysis by Mills *et al.*	IDDA
Juvenile	juv
Knotted	k, kn
Large	lge, lg
leafy material	lm
Light	lt
Listed to contain	ltc
Manila	man
Material	mat, mat'l
Metal	met
microscopic examination	micro
multi-colored	mc, multi
Mushrooms	mush
negative Result for a test	-, circled -, neg
nitroprusside color test	np
no change	nc
no color reaction	ncr
no reaction	nr
no significant reaction	nsr
not analyzed	na, not anal., nap, nt, DIRP
not opened	n/o, no
off-white	off-wh, ow
Package	pkg
Packet	pkt
Paper	pap
Pentane	pent
physical identification code for brand name product	PIB, PIBr
physical identification code for generic product	PIG
physically identified with references	PID

Physicians Desk Reference	PDR
placed in zip(s) in lab	PIZIL
plant material (only)	pm
Plastic	p, plas, pls, plst
Powder	pwd, pW, pwdr
Precipitate	ppt
Prescription	Rx
Prince William County	Pr W
Probable	prob
Purple	prpl, pur, pl, ppl
quantity insufficient	qns
Reaction	rxn
Remaining	rem
repackage(d)	repkged
Residue	res
Returned	ret
rolling papers	roll pap
sample	sam
schedule	Sch, sched
schedule I	CI
schedule II	CII
schedule III	CIII
schedule IV	CIV
Schedule V	CV
schedule VI	CVI
Sealed	"circled" word or acronym, s (in front of another code)
smoking device	sd, smok dev, s-d
Solid	sol
solid material	sm
Solvent	solv
Strong	str
Substance	subst
Synthetic Cannabinoid	syn can
Syringe	syr
tablet(s)	tab(s)
twist-tied	tt
Very	v
Violet	vio
Volume	vol
Weak	wk
Which contained(s)	w/c
White	wh, wht
Wrapper	wrap
Yellow	y, yell, yl
Ziplock	zip, z

Drug Name	Abbreviation
3,4-Methylenedioxyamphetamine	MDA
3.4-Methylenedioxymethamphetamine	MDM, MDMA
6-Monoacetylmorphine	MAM, 6-MAM
Acetaminophen	AC, APAP
Alprazolam	AL
Amphetamine	A
Aspirin	ASP
Benzocaine	BNZ, BENZO
Bufotenine	BUF
Buprenorphine	BUP
Benzylpiperazine	BZP
Caffeine	CAF
Cannabidiol	CBD
Cannabinol	CBN
Clonazepam	CZM
Cocaine	CO, COC
Cocaine base	CB, CFB
Cocaine Hydrochloride	COH
Codeine	COD
Diazepam	D
Dimethyltryptamine	DMT
Ephedrine	EP
Fentanyl	F
Gabapentin	GAB
Gamma-butyrolactone	GBL
Gamma-hydroxybutyric acid	GHB
Heroin	HER
Hydrocodone (Dihydrocodeinone)	HC, DI
Hydromophone	HM
Ibuprofen	IB
Insufficient for analysis	IFA
Insufficient for identification	IFI
Ketamine	KET
Lidocaine	LIDO
Lorazepam	LO
Lysergic acid diethylamide	LSD
Marijuana	MJ
Methadone	MN, MDN
Methamphetamine	M, METH
Methylphenidate	MPH
Miscellaneous	MIS
Morphine	MO
Nicotine	NIC
No controlled substance identified	NCI

No controlled substances found	NCS, NCSF
Oxycodone	OC, OXY
Oxymorphone	OX
Pethidine (Meperidine)	PE
Phencyclidine, liquid	PCL
Phencyclidine	PCP
Phenylpropanolamine	PPA
Previously analyzed	PRE
Procaine	PR, PRO
Pseudoephedrine	PSE, PSU
Psilocybin	PSB
Psilocyn	PS
Quinine	QUI
Synthetic Cannabinoid	Syn Can, Syn Cann
Δ-9 Tetrahydrocannabinol	THC
Tramadol	TRA
Trifluoromethylphenylpiperazine	TFMPP